THE DEVELOPMENT OF ALTERNATIVE STRATEGIES FOR USING CHEMICAL OXIDIZING AND REDUCING AGENTS IN FLOURS AS A MEANS OF CONTROLLING GLUTEN STRENGTH PRIOR TO DEVELOPING DOUGH

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ABSTRACT

The overall goal of this research was to understand the dough handling parameters and baking performance effects of different commercial enzymes in relation to commonly used chemical oxidizers to enhance flour quality in the baking industry. Thus, evaluate the enzymes viability to create 'cleaner-labeled' wheat flour and bread, as they are considered processing aids. For that, the inter-relationships between wheat grain and flour quality parameters, dough rheology, and baking of different commercially grown Western Canadian wheat cultivars was examine. In addition, the research also allowed to better understand the Canadian Western wheat modernization class in relation to the baking industry.

In Chapter 3, a range of commercially grown Canadian spring wheat (*Triticum aestivum L.*) cultivars (n = 25) within different wheat market classes were investigated to understand the inter-relationships between wheat quality, grain and flour composition, and dough rheology. The cultivars varied in proximate composition which in turn directly impacted their dough handling parameters. Micro-doughLAB absorption was positively correlated with protein content, grain hardness, wet gluten and dry gluten content, and was negatively correlated with the gluten performance index. Significant correlations ($p<0.01$) between shear rheology parameters were also found with gluten properties. Protein and gluten properties in particular, significantly impacted dough strength measurements, therefore, cultivars displaying stronger gluten strengths might result in dough with better dough handling properties. From this set, five cultivars were selected based on their overall performance and market class representatively to have weak, intermediate, and strong dough strength.

In Chapter 4, the quality parameters (i.e., proximate composition, flour yield, gluten properties) and dough strength (i.e., empirical and fundamental rheology) of different wheat cultivars ranging in gluten strengths from weak (Harvest), intermediate (Lillian, CDC Plentiful and Stettler) to strong (Glenn) were analyzed with the addition of chemical oxidizers (i.e., ascorbic acid, azodicarbonamide) or commercial enzymes (i.e., glucose oxidase and fungal xylanase). The use of enzymes is attractive to the baking industry as an alternative to chemical oxidizers as dough strengtheners, resulting in cleaner label products (i.e., fewer ingredients). Glenn presented better overall quality attributes compared to the other cultivars, and responded well to additives, especially glucose oxidase, which significantly improved dough strength. Glucose oxidase also improved the dough handling of weaker cultivars. Thus, the addition of enzymes gave dough similar rheological properties to dough prepared with chemical oxidizers. To further analyze the enzymes performance over chemical oxidizers through the set of five

cultivars, a baking trial was crucial to understand the performance of enzymes in actual baking in regards to dough and bread crumb structure.

In Chapter 5, the effect of chemical oxidizers and enzymatic treatments on the baking quality of breads formulated with five Canadian spring wheat cultivars were investigated. Dough and bread properties (mixing time, oven rise, loaf volume, crumb firmness and C-cell parameters) were analyzed as a function of wheat cultivar (Glenn, Harvest, Lillian, CDC Plentiful, and Stettler), additive-type (ascorbic acid, azodicarbonamide, glucose oxidase, and fungal xylanase) and concentration. Overall, the cultivar Glenn had improved baking performance relative to the other cultivars, regardless of the additive and additive concentration. On the other hand, Stettler showed poorer baking quality and performance even with the addition of oxidizers and enzymes in relation to the control. The concentration of additive was found to have little or no effect on improving baking properties within each cultivar. Enzymes had similar or better performance than oxidizers in most cases.

In Chapter 6, the effect of the concentration of a reducing agent (L-cysteine), commonly used in the baking industry, on the rheology and baking performance of doughs prepared using five western Canadian spring wheat cultivars was studied. The relationship between the production time and quality of bread is crucial in the bakery industry. Therefore, reducing agents can be used in stronger wheat cultivars as means to improve efficiency of production (i.e., lower mixing time) and result in equal or higher quality bread loaf (i.e., loaf volume). The addition of L-cys resulted in a significant ($p<0.05$) decrease in dough strength and handling properties, where stronger gluten strength wheats were less effected by addition and had improved dough handling properties, loaf volume, and softer crumb structure. The addition of L-cys to wheat flours reduced mixing time up to 47%, increased loaf volume (up to 9%), and elasticity of the products, those characteristics are desired to increase the efficiency of the automated processes for bread products.

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LIST OF SYMBOLS AND ABBREVIATIONS

|G*|	Complex modulus
AA	Ascorbic Acid
AACCI	American Association of Cereal Chemists International
ABS	Absorption
ADA	Azodicarbonamide
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AX	Arabinoxylan
BPR	Bandwidth at peak resistance
CGC	Canadian Grain Commission
CNHR	Canada Northern Hard Red
CPSR	Canada Prairie Spring Red
CWES	Canada Western Extra Strong
CWHWS	Canada Western Hard White Spring
CWRS	Canada Western Red Spring
CWSP	Canada Western Special Purpose
ddH_2O	Deionized and distilled water
DDT	Dough development time
DG	Dry gluten
FN	Falling number
G’	Shear storage modulus
G”	Shear loss modulus
GI	Gluten index
Gox	Glucose oxidase
GPI	Gluten performance index
HI	Hardness index
J_{el}	Relative elasticity
J_{max}	Creep compliance
L-cys	L-cysteine
LV	Loaf volume
MDT	Mixograph development time

MTI	Mixing tolerance index
NSP	Non-starch polysaccharides
PDR	Peak dough resistance
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Semicarbazide
SRC	Solvent retention capacity
STA	Stability
tan δ	Loss tangent
USDA	The United States Department of Agriculture
WG	Wet gluten
WIP	Work input to peak
Xyl	Xylanase
γ	Strain
τ_0	Shear stress

1. INTRODUCTION

1.1 Overview

Wheat (*Triticum aestivum* L.) is an important industrial crop that can be grown under heterogeneous types of weather, elevation or soil. In Canada, wheat is the major crop produced mainly in the southern areas of the Prairie Provinces of Manitoba, Saskatchewan and Alberta. The Canadian Western Red Spring (CWRS) wheat crop encountered market challenges over the years, due to weaker gluten properties as a result of environmental conditions, crop diseases (such as fusarium head blight, FHB), insect pressure (such as wheat midge) and crop management. This contributes to poorer dough mixing properties and end product quality. In early 2015, the Canadian Grain Commission started a wheat modernization process that aimed to remove weaker gluten cultivars from the Canada Western Red Spring (CWRS) and Canada Prairie Spring Red (CPSR), based on class quality, consistency and end-use performance. In August 2018, two new wheat classes were implemented, such as Canada Northern Hard Red (CNHR) and Canada Western Special Purpose (CWSP). Initially, 29 cultivars were re-assigned, being 25 CWRS and 4 CPSR. Thus, examining the relationship of dough strength to processing is of importance in designing breeding strategies for the next decade.

To overcome deficiencies in wheat quality, additives such as chemical oxidizers, (i.e., azodicarbonamide, potassium bromate and iodate, peroxides, and L-ascorbic acid) can be used in the milling and baking process. These chemicals oxidize the sulphydryl (-SH) groups of gluten proteins, forming disulfide linkages, which act to improve the strength and handling properties of the dough of weaker flours. However, some of these additives have been associated with causing some forms of cancer, and thus are now banned for use in Europe and are under review in other countries. In addition to the possible negative effects of chemical oxidizers, the market consumer trend for cleaner label products also emphasizes the importance finding alternatives. Enzymes have shown to represent a potential substitute to chemicals, as they can act in a similar manner as oxidizers by promoting the formation of disulfide linkages among the gluten proteins, increasing dough strength and improving dough handling properties.

In addition, because they are proteins and, thus, generally denatured during baking, enzymes are considered processing aids and are not required to appear on product labels.

In order to tackle the overall goal, twenty-five commercially grown Canadian Western selected wheat cultivars representing different gluten strengths (i.e., weak, medium, and strong) and wheat classes were evaluated for their potential to mediate the effects of replacing chemical oxidizers in flour for bread making. This research supported the wheat value chain as the milling and baking industries undergo product reformulations in response to consumer trends and government regulations. Therefore, the thesis research looked to develop cleaner labels using two approaches *i*) by examining a range of wheat cultivars of varying gluten strengths in relation to the new wheat class modernization; and *ii*) by the addition of enzymes in order to remove industries' reliance on chemical oxidizers and reducing agents.

1.2 Objectives

- To study the inter-relationships between wheat and flour quality parameters and composition, and dough rheology of a set of wheat cultivars (n = 25) representing Western Canadian wheat classes, such as Canada Western Red Spring (CWRS), Canada Northern Hard Red (CNHR), Canada Western Hard White Spring (CWHWS), Canada Prairie Spring Red (CPSR), Canada Western Special Purpose (CWSP), and Canada Western Extra Strong (CWES).
- To evaluate the effect of chemical oxidizer-type (azodicarbonamide and ascorbic acid) and oxidizer-concentration on the mechanical properties of dough and bread making performance in a range of five wheat cultivars differing in gluten strength (from weak to strong).
- To study the role of enzymes (glucose oxidase and fungal xylanase) and enzyme-concentration as a replacement for chemical oxidizing and reducing agents on dough handling and bread loaf quality properties in relation to cultivar-type, additive-type, and additive-concentration.
- To evaluate the effect of reducing agent L-cysteine as a reducing agent and its performance on baking and dough handling properties for range of five wheat cultivars differing in gluten strength (from weak to strong).

1.3 Hypotheses

- Stronger cultivars will be less affected by the addition of chemical oxidizers or enzymes, and will have positive response to reducing agents in comparison to weaker or intermediate cultivars;
- As the concentration of enzymes and chemical oxidizers increases, the dough will become more elastic with improved dough rheology properties and baking parameters;
- Glucose oxidase will have similar effect on dough handling to ascorbic acid;
- The addition of fungal xylanase will promote protein (gluten) aggregation, due to more water available in the media for protein network formation, resulting in higher loaf volumes and uniform crumb structure;
- Additive-concentration will play an important role in both baking performance and dough handling;

2. LITERATURE REVIEW

2.1 Wheat and wheat quality

Wheat (*Triticum aestivum* L.) is a versitile commercial cereal crop largely grown due to its suitability to diverse types of weather, elevation, and soil (Liu et al., 2015; Enghiad et al., 2017). The world wheat production in 2016/17 reached 745 million tons, with the European Union, Russia and United States being the major producers (CIGI, 2016). In Canada, wheat is the major cereal crop produced, with a production of ~32 million tons over 9.4 million hectares (2016/17 crop year). The southern areas of the Prairie Provinces of Manitoba, Saskatchewan and Alberta account for almost 95% of all Canadian wheat produced (IGC, 2016). Due to its high overall quality and dynamic processing ability, approximately, 70% of the total wheat production is designated for exportation to countries such as China, Japan and Mexico (IGC, 2016).

2.1.1 Wheat grain composition

The common *Triticum aestivum* L. is a hexaploid with three genomes, A, B, and D (i.e., it has six copies of its seven chromosomes, 42 chromosomes total) wheat with endosperm texture that ranges from very soft to hard. On the other hand, the tetraploid (two genomes, A and B) durum wheat (*T. turgidum* L. *ssp*. durum) has the hardest kernels of all wheat cultivars (Delcour and Hoseney, 2010a). The wheat grain kernel (Figure 2.1) varies in length from 4-8 mm, weighs from 35-55 mg, and ranges in color from white to red to black. These variations in size, weight and color are the result of different cultivars and the kernel location in the wheat (head or spike) (Cornell, 2012). The wheat kernel is composed of three main parts: the endosperm (~80-85%), which contains mostly starch and proteins; the germ (~3% germ), composed mostly of lipids and proteins; and the bran (~13-17%), containing mainly dietary fiber (Šramkováa et al., 2009; Pauly et al., 2013; Liu et al., 2015). The germ is located at the end of the kernel and is rich in protein (~25%), lipids (8-13%) and vitamin E. The outer layer of the kernel, the pericarp, is comprised of multiple layers (~50 μm thick) and plays a role in protecting the grain by acting as a barrier. The endosperm is comprised of 70 to 82% of starch ("starchy endosperm") with an outer layer called aleurone. Apart of the high carbohydrate

concentration, the endosperm accounts for up 70% of all the wheat grain proteins (e.g., albumins and globulins) responsible for gluten formation. The bran is mainly comprised of water-insoluble fiber, cellulose, pentosans, xylose, and arabinose (Šramkováa et al., 2009; Cornell, 2012; Mandarino, 2013).

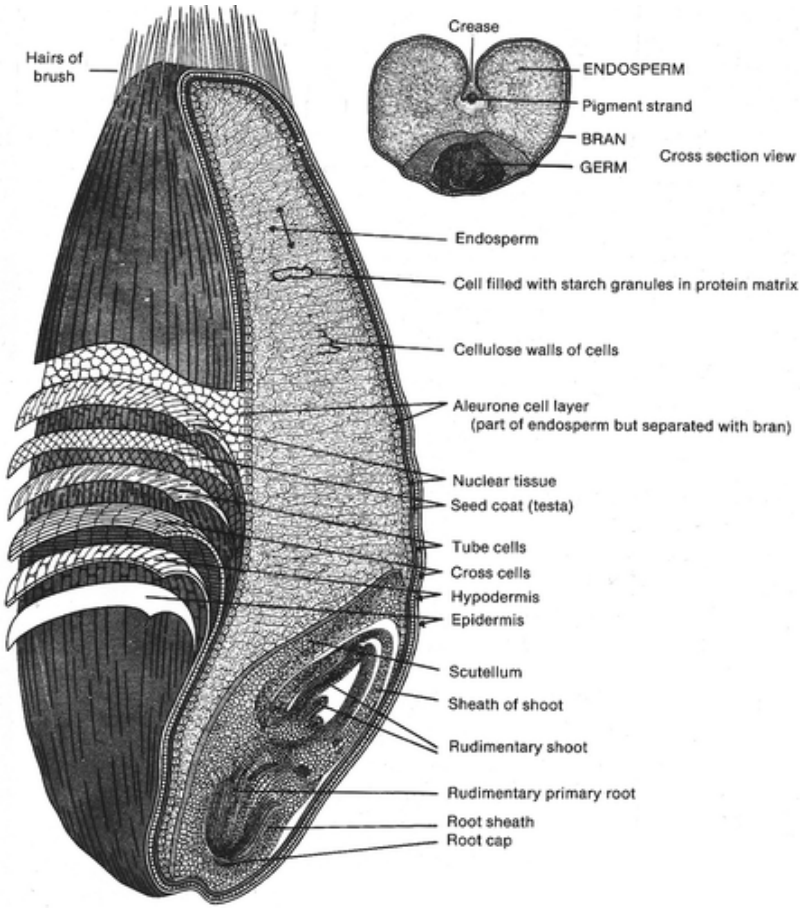

Figure 2.1. Longitudinal and cross sections of a wheat kernel (Delcour and Hoseney, 2010a).

Wheat starch

In wheat, the carbohydrates represent almost 75% of the kernel composition, being classified as soluble (starch) and insoluble (cellulose, hemicellulose, and pentosans) (Whang et al., 2018). Starch is an important natural polysaccharide formed in the amyloplasts, being the major energy storage in wheat. It is composed of linear or slight branched amylose (α (1–4) linkages) and branched amylopectin (α (1–4) linkages and α (1–6) linkages), which can easily be converted into glucose (Majzoobi et al., 2011; Zhao et al., 2019). Amylopectin consists in three types of chain, A, B, and C-type. The A-type granules are comprised of glucose α-1→4 linkages and constitute the majority of the starch in the grain by weight. In contrast, B-type granules chains are comprised of α→1,4 and α→1,6 glucose linkages and comprise up to 99%

of granules in number (including C-type). The C-type granules are made up of glucose with a α-1→4 and α-1→6 linkages, and a reducing group. These different conformational structures between amylose and amylopectin result in different properties and functions (Damoraran, 2007; Denardin and da Silva, 2009; Delcour and Hoseney, 2010d). The molecular structure and difference in the starch composition can be associated with baking quality of wheat flour (i.e., degree of fermentation and staling) (Zhang et al., 2010). This difference can occur due to genotype variation, but also, depending on grain position in the spike (i.e., superior and inferior) (Zhang et al., 2010; Whang et al., 2018).

Wheat proteins

Wheat has a particular capacity of being the only cereal to form a three-dimensional viscoelastic dough when mixed with water due to its unique protein composition (Bockstaele et al., 2008). The total protein content on wheat grain varies from 10% to 18% depending on the wheat cultivar (genotype), environment conditions and crop management techniques (Šramkováa et al., 2009). Usually, proteins are classified into the Osborne fractions, including albumins (soluble in water), globulins (soluble in salt solution), prolamins (soluble in alcohol) and glutelins (soluble in dilute acids) (Schalk et al., 2017). In wheat, albumins and globulins are high in three essential amino acids, such as lysine, tryptophan, and methionine, and are mainly concentrated in the aleurone cells, bran, and germ, with a lower concentration in the endosperm (Jiang et al., 2008). In contrast, prolamins and glutelins are concentrated in the endosperm, known as gluten-forming proteins (gliadin and glutenin) (Delcour and Hoseney, 2010d; Balakireva and Zamyatnin, 2016). Because gluten has low content of charged amino acids, such as lysine, arginine, glutamine, and asparagine residues (<10% of the total amino acid residues) it is insoluble in water (Damodaran, 2007). In addition, the high concentration of cysteine and cystine residues (2–3 mol% of total amino acid residues) in gluten proteins undergo sulfhydryl–disulfide interchange reactions, resulting in extensive polymerization of these proteins helping to form its viscoelastic characteristics (Damodaran, 2007). Therefore, gluten provides dough with extensibility, viscosity, elasticity, cohesiveness and contributes to its water absorption capacity (Uthayakumaran et al., 1999; Joye et al., 2009).

Together, the gluten-forming proteins gliadins and glutenins represent 80–85% of the total proteins of wheat flour (Figoni et al., 2008). Glutenins are heterogeneous polypeptides with molecular weights ranging from 12,000 to 130,000 Da, which can be classified as high molecular weight (Molecular weight > 90,000 Da, HMW) and low molecular weight

(Molecular Weight < 90,000 Da, LMW) glutenins. These polypeptides are present as polymers joined by disulfide cross-links (e.g., reaction with cysteine Cys-SH), thus, due to their ability to polymerize extensively via sulfhydryl–disulfide interchange reactions, glutenins contribute greatly to the elasticity of dough and very little to dough extensibility (Damodaran, 2007; Delcour and Hoseney, 2010d; Schalk et al., 2017). In contrast, gliadins are a heterogeneous mixture of monomeric gluten proteins with molecular weights ranging from 30,000 to 80,000 Da. Four different groups of gliadins can be found, including: α-, β-, γ - and ω-types. The α-type contains six cysteine residues whereas the γ-type contains eight cysteine residues. These types of proteins are part of the S-rich group of prolamins and have similar structures. α- and γ-Gliadins contain a relatively high composition of cysteine and methionine, but few glutamine, proline and phenylalanine residues. In contrast, the ω- type gliadins are considered sulphur-poor prolamins, due to a lack of cysteine residues. The disulfide bonds are intramolecular buried in the interior of the protein, because of that they do not take part in sulfhydryl–disulfide interchange reactions with other proteins. Due to these characteristics, gliadins confer high extensibility and low elasticity to the dough, i.e. the dough from isolated gliadins is viscous, but not viscoelastic in nature (Damodaran, 2007; Delcour and Hoseney, 2010d; Balakieva and Zamyatnin, 2016). Therefore, the glutenin polymer structure, size distribution, and subunit composition, and the gliadin/glutenin ratio strongly affect gluten quality and, as consequence, the breadmaking potential of wheat flour (Joye et al., 2009).

Other constituents

Even though starch, proteins, and water account for most of the compositional part of wheat flour (90-95%), minor constituents, such as non-starch polysaccharides (2-3%), lipids (~2%), vitamins and minerals (~1%), play an important nutritional and processing role in wheat (Delcour and Hoseney, 2010d). The non-starch polysaccharides (NSP) are mainly composed of cellulose, arabinoxylan (AX), β-D-glucan, and arabinogalactan. Up to 75% of the dry matter weight of wheat endosperm cell wall is composed of NSP, predominantly AX (Ahmad et al., 2014; Delcour and Hoseney, 2010d). AX can be classified into soluble or water-extractable arabinoxylans (WE-AX) and insoluble or water-unextractable arabinoxylans (WU-AX) (Ahmad et al., 2014). Because of their ability to absorb water (up to four times their weight), AX can disturb the gluten network formation by competing for water, thus, affecting dough formation and bread quality (Döring et al., 2015). However, the amount, structure, and functional properties can vary according to the wheat cultivar, for instance, the molecular weight and distribution, branching pattern, extractability with water interaction with other cell

wall components such as lignin or cellulose (Goesaert et al., 2005; Delcour and Hoseney, 2010d; Garófalo et al., 2011). Cellulose is a structural polysaccharide in plants with a simple structure composed of β-1,4-linked D-glucose units. Due to its linear configuration, cellulose associates strongly with itself becoming insoluble, for this reason the polymer resistant to many organisms (e.g., humans cannot degrade it due to lack of the cellulase enzyme). Hemicellulose is the nonstarch and noncellulosic plant polysaccharide, which can vary composition from single sugars to pentoses, hexoses, and phenolics. In wheat, the predominant hemicellulose is arabinoxylan, which can be water-extractable (WUAX) or water-unextractable (WUAX). Both are technologically important as they can bind up to 10 and 5 times their own weight in water, respectively, influencing gluten hydration and formation during breadmaking (Delcour and Hoseney, 2010d; Bock, 2015).

Lipid molecules are distributed through the wheat kernel as structural components in oil-rich tissues, such as aleurone, scutellum and embryonic axis (Morrison, 1978). Even though lipids represent only a small fraction of the wheat kernel and derived flour (<3%; with ~28% concentrated in the germ), they have an important role in determining wheat quality and the texture of the final products (Šramkováa, 2009; Delcour and Hoseney, 2010d). During the dough development, lipids can form protein-lipid and starch-lipid complexes influencing dough properties (Gerits et al., 2014b). Most of these lipids are surface active (phospholipids) which have a role stabilizing the gas bubble's surface during dough formation (along with the protein), and helping to improve bread volume and dough stability (Cornell, 2012).

2.1.2 Canadian Western wheat classification

Wheat classification is mainly based on four major criteria, including kernel texture (hard and soft), bran color (red or white), growth habit (spring or winter), and grain protein content (gluten strength) (Carson and Edwards, 2009). The kernel texture represents the physical resistance of the wheat kernels to crushing during the milling process. Different methods can be used to describe kernel texture (i.e., grain hardness), such as dynamic methods wheat hardness index (WHI), particle size index (PSI), and pearling resistance index (PRI), or directly with the use of near-infrared technique (NIR) spectroscopy or Perten Single-Kernel Characterization System (SKCS) (Salmanowicz et al., 2012). The properties of wheat flour can also be affected based on grain hardness, for instance, sifting capacity, starch damage during milling, susceptibility to amylolytic enzymes, improved fermentability, water absorption of flour, and improved baking value of produced bread, therefore, being an important criterion for determining end use of various wheat classes (Martin et al., 2001). The grain color and

appearance significantly affect the market value of wheat, where misclassification of color classes result in poor grain quality and a loss of monetary value. The color is impacted by the environment and management practices during the crop year, in addition to grain protein content, hardness, virtuousness, and kernel size and shape which may, also, contribute to variation in visual grain color grading (Peterson et al., 2001).

In Canada, a wide variety of wheat is grown, each one with its own unique properties for applications in the industry and focus in the exportation market. Until August 2018, the Western Canadian wheat classes was composed of eight milling sub-classes (Grain Canada, 2015). The two largest classes of wheat grown are Canada Western Red Spring (CWRS) and Canada Western Amber Durum (CWAD). By far, the largest and most exported market class within bread wheat is the CWRS typically used for the production of high-volume pan and crusty bread. CWRS cultivars have excellent milling quality, and the flour is characterized by high farinograph water absorption (between 64 to 70%), and well-balanced gluten strength suitable for both, no-time and long-fermentation sponge and dough baking process (Carson and Edwards, 2009). The CWRS has an annual average production of, approximately 15 million tons. The high quality characteristics (i.e., high protein, 12.8-14.8%, and gluten strength) in addition to the dynamic processing characteristics give this class the flexibility to be exported to over 60 markets (Dexter et al., 2006; Canadian Wheat, 2018). Overall, CWRS cultivars have high test weight (~82 kg/hL), good milling performance (i.e. flour yield (~75%), low ash content and high brightness), and high resistance to pre-harvest sprouting (Wang et al., 2003; Dexter et al., 2006; Canadian Wheat, 2018). The main quality parameters for CWRS wheat are presented on Table 2.1 for 2016's crop in Western and Eastern Canada Prairies. The second largest grown cultivar in Western Canada is the Canada Western Amber Durum (CWAD), which account for an average production of ~5 million tons production/year. This wheat class has high weight to ensure high semolina yield (65.4 to 66.7%) and quality (i.e. must meet maximum ash and/or minimum brightness specifications) and it is target for mainly for pasta production. Furthermore, CWAD has high protein content (13.4 to 13.6%), as it is directly associated with pasta texture (Dexter et al., 2006; Canadian Wheat, 2018). Some minor classes of wheat produced in Western Canada, such as Canada Western Extra Strong (CWES), Canada Western Red Winter (CWRW), Canada Prairie Spring Red (CPSR), Canada Prairie Spring White (CPSW), Canada Soft White Spring (CWSWS), and, as of 2018, Canada Northern Hard Red (CNHR), and Canada Western Special Purpose (CWSP).

The Canadian wheat market class system has evolved over time to handle changes in the end-use quality types demanded by both, the domestic and international industry (McCallum and DePauw, 2008).

Table 2.1. Quality parameters of Canada Western Red Spring Wheat, No. 1, crop 2016 (Canadian Grain Commission, 2016a).

Quality parameter[1]	2016			
	Western Prairies[2]		Eastern Prairies[2]	
Wheat				
Test Weight, kg/hL	80.3		81.2	
Weight Per 1000 Kernels, g	36.8		33.8	
Protein Content, %	13.7		13.8	
Protein Content, % (dry matter basis)	15.9		16.0	
Ash Content, %	1.38		1.42	
Falling Number, sec	410		435	
Particle Size Index, %	55		51	
Milling Flour Yield				
Clean wheat basis, %	75.7		75.6	
0.50% Ash basis, %	79.2		78.6	
Flour				
Extraction, %	**74%**	**60%**	**74%**	**60%**
Protein Content, %	13.0	12.7	12.9	12.5
Wet Gluten Content, %	36.5	35.3	35.5	34.8
Gluten Index, %	89.0	Nd	92.0	nd
Ash Content, %	0.43	0.39	0.44	0.39
Starch Damage, %	8.0	8.3	8.6	8.9
Amylograph Peak Viscosity, BU	510	560	640	675
Farinograph				
Absorption, %	65.5	65.0	64.9	64.3
Dough Development Time, min	5.50	6.50	5.50	6.75
Stability, min	7.5	9.0	7.5	9.5
Mixing Tolerance Index, BU	25	30	30	30
Baking (Canadian Short Process)				
Absorption, %	70	69	68	68
Mixing time, min	4.4	4.4	4.2	4.3
Mixing energy, W-h/kg of dough	10.8	11.2	10.0	9.9
Loaf volume, cm^3/100 g flour	985	1005	1010	1015

[1.] Data are reported on a 13.5% moisture basis for wheat and a 14.0% moisture basis for flour.
[2.] Western Prairies includes BC, AB, western SK; Eastern Prairies includes eastern SK and MB

In early 2015 the Canadian Grain Commission started a wheat modernization process that aimed to remove weaker gluten cultivars from the Canada Western Red Spring (CWRS) and

Canada Prairie Spring Red (CPSR), based on class quality, consistency and end-use performance (Japp, 2019). In August 2018, two new wheat classes were implemented, such as Canada Northern Hard Red (CNHR) and Canada Western Special Purpose (CWSP). Initially, 29 cultivars were re-assigned, being 25 CWRS and 4 CPSR (Canadian Grain Commission, 2018). Within the 25 cultivars selected for this research, Harvest, Lillian, McKenzie, Pembina, and Unity were reclassified from CWRS to CNHR and CDC Kinley and Pasteur from CWRS to CWSP (CGC, 2019c).

As the main purpose of the modernization was to ensure that new cultivars would meet requirements for milling performance, dough strength, protein quantity and product quality, the cultivar checks for CWRS trials were also changed. The Prairie Recommending Committee for Wheat, Rye and Triticale (PRC-WRT) proposed the use of Glenn (as high maximum gluten strength) and Carberry (as minimum level of gluten strength) for check cultivars for the Central and Western Trials, removing Unity VB and Lillian as quality checks. Therefore, to increase the uniformity and consistency in the registration process within the CWRS class (Canadian Grain Commission, 2015a,b). On the other hand, the CPSR would have Glenn as check cultivar as high end for good extensibility and correct protein, and AAC Foray as the moderate-high gluten strength check, HY537 as the moderate-low gluten check, and 5700PR as the minimum gluten strength check, thus, removing cultivars with lower gluten strength than 5700PR from the CPSR (Canadian Grain Commission, 2015a,b).

2.1.3 Wheat Flour

Wheat (*Triticum aestivum*, L) flour is the main product derived from milling the wheat grain (Figoni, 2008). It is the basic ingredient for several bakery products in different cultures assuming economic, nutritional and religious significance (Shewry and Hey, 2015). During milling, the wheat endosperm is separated from other wheat-grain components (germ and bran) to meet a standard particle size and form wheat flour (Wrigley et al., 2006). Depending on the milling process, the grain can go through different forces, such as compression, shearing, crushing, cutting, friction and collision (Voicu et al., 2013). The commonly used techniques for grinding are the stone mill (SM), roller mill (RM), ultra-fine mill (UM), and hammer mill (HM) (Liu et al., 2015).

Roller Milling (RM)

The RM process is a mechanical process to separate the wheat grain endosperm from the bran and germ into flour through different sets of rolls and sieves (Pauly et al., 2013; Liu et

al., 2015). Mills with this process are usually equipped with a grinding machine (roller mill), a machine for sifting and sorting of the resulted milling fractions (plansifter compartment) and, eventually, a machine for the conditioning of semi-final product (bran finisher) (Voicu et al., 2013). The process accounts for a series of steps from reception and storage, cleaning, tempering (or conditioning), and to the final milling process itself. The wheat grain is tempered by a combination of heat, water and rest, as means to soften the endosperm and plasticize the bran, becoming less susceptible to fragmentation (Delcour and Hoseney, 2010c). Different grain moisture is desired, depending on the wheat hardness, varying from 14.5% (soft) to >17% (durum wheat) (Delcour and Hoseney, 2010c; Pauly et al., 2013). During the milling process, factors such as arrangement of the rollers, differential speed, distance between the rollers, flutes profile and position can greatly influence the final flour quality (i.e., particle size, bran contamination, and flour yield) (Voicu et al., 2013). The RM method can be more economical and flexible, have less heat output during production and, as result, less destruction to flour components, such as protein degradation. Also, the by-product bran and germ, can go through post-milling processing to be commercialized (Liu et al., 2015).

2.1.4 Wheat grain and flour quality

Wheat processing quality is strongly dependent on its physical condition and composition (Nuttall et al., 2017). In Canada, the wheat quality is overseen by the Canadian Grain Commission (CGC) by a variety registration and quality assurance system (cleaning, testing and segregation), which assures the quality based on industry needs and fundamental principles, such as reliable supply, safety, cleanliness, uniformity and consistency, and superior processing performance (Dexter et al., 2006). Within Canadian wheat cultivars, CWRS has the reputation for superior quality and uniformity due to tight tolerances on the presence of degrading factors influencing processing (Preston et al., 2001).

The characterization of the wheat grain and flour, and the flour's rheological properties is fundamental to predict the processing behavior and, consequently, determining the quality of the final wheat derivate products (e.g., crumb structure and loaf volume) (Song and Zheng, 2007). Flour quality tests predict or determine the milling efficiency and end-product applicability. These tests are subdivided into different groups to determine its basic constituents (i.e., proximate analysis, such as moisture, protein, lipid and ash content), processing parameters (i.e., color, wet gluten, gluten index, starch damage, particle size, amylograph, and falling number), and dough strength (i.e., mixograph, alveograph, farinograph, and shear rheometry) (Preston and Williams, 2003). In addition, gluten content, composition and

characteristics, indicates whether the gluten formed is weak, intermediate or strong. In addition, for characterizing dough rheology, empirical rheological tests, such as farinograph, mixograph and extensograph, and fundamental rheological measurements, such as oscillatory frequency sweep and creep-recovery test, are frequently used (Song and Zheng, 2007; Jekle and Becker, 2011).

Grain quality and, hence, flour quality are influenced by four main factors, such as genotype, management practices, and environmental conditions (Nuttall et al., 2017). The environmental conditions have direct and significant impact on different classes and genotypes of wheat, parameters such as useful-heat accumulation and water stress are predominant factors influencing grain development (Finlay et al., 2007). For instance, drought stress is one of the leading constraints to wheat production, where phenotyping continues to be largely used as criteria for screening breeding on drought adaptive and constitutive morphophysiological (i.e., yield and its components) (Mwadzingeni et al., 2016). In Canada, due to the vast size of the wheat growing region a big variation in temperature and precipitation can occur, consequently there is a wide range in wheat quality outcomes across the Western provinces (Bhatta et al., 2017).

For Surma et al. (2012), genotype was found to have a major influence on grain hardness, protein content, wet gluten and sedimentation value. Whereas, the environment had equal importance as genotype for starch content, alveograph parameter and hectoliter weight. In addition, Battha et al. (2017) showed significant correlations between nitrogen (N), environment, and seeding rate with protein content and dough strength. A higher supplementation of N resulted in increased grain protein content, as it was more available to the plant at the critical stage of grain formation. On the other hand, a higher seeding rate irrespective of N treatment resulted in lower grain protein content, due to competition at the higher plant densities, but higher grain yields. Furthermore, studies have shown that environment and cultivar type had significant effects on farinograph and dough mixing parameters. For instance, Finlay et al. (2007) presented differences between cultivar (C), environment (E), and C x E, where environmental quality parameters variation was much larger than genotype related variation (1.3 to 3.5 times, respectively), agreeing with results presented by Surma et al. (2012).

The post-harvest grain management is another determinant factor for wheat quality as the grain is susceptible to large climatic variation throughout the year, impacting the temperature, humidity, and overall gas exchange in the storage silos (Manandhar et al., 2018). The main form of wheat storage in Canada is on-farm steel silos (Dexter et al., 2006). A poor

post-harvest management can lead to microbial growth causing innumerous effects towards grain quality loss, such as discoloration, heat generation, utilization of carbohydrates, lipids and proteins degradation (altering digestibility), production of volatile metabolites (off-odors), loss of germination and baking (Magan and Aldred, 2007). In addition, filamentous fungal spoilage organisms, such as *Fusarium* head blight, can produce mycotoxins (deoxynivalenol, DON) (Fernandez et al., 2005). In CWRS, the tolerance levels of mycotoxins established for *Fusarium* is 0.25% up to 2.0% in CWRS (Dexter et al., 2006; Delcour and Hoseney, 2010a). In addition, dockage such as weed seeds and stems, chaff, straw, or grain other than wheat), must be removed from wheat to meet commercialization standards. Dockage free wheat results in less cleaning steps prior milling, improves storage stability, and diminish exportation restrictions (Dexter et al., 2006).

2.2. Bread making

Bread is one of the oldest and most widely consumed foods in the world contributing, substantially, to the daily intake of carbohydrates, dietary fiber, minerals and B vitamins (Joye et al., 2009). Bread is traditionally made from cereal flours, particularly from wheat, where the gluten proteins (gliadin and glutenin) combine to give a highly viscoelastic dough material (Mondal and Datta, 2008; Mucahit, 2012). The dough is a complex material of a variety of ingredients and phases (gases, solids, and liquids). The essential ingredients in bread include wheat flour, salt (NaCl, sodium chloride), water and yeast (*Saccharomyces cerevisiae*). Whereas, non-essential ingredients include sugar (role: energy, color, and flavor), enzymes (role: bread quality and dough strengthening), dairy products (role: enhances nutrition and color), shortening or fat (role: acts as a softener and dough plasticizer), emulsifying agents (role: plasticizer, softener and bubble stabilizer) and improvers (role: shelf-life and bread quality) (Liu and Scanlon, 2003; Mondal and Datta, 2008; Mucahit, 2012).

2.2.1 Essential ingredients in dough

Sodium chloride (NaCl). Although NaCl is not in significant amounts in dough formulations (~1-2%), it plays a magnitude of roles in dough/bread contributing to flavor, improving dough strength, modulating yeast fermentation, reducing water activity, inhibiting microbial growth and extending product shelf life (Mondal and Datta, 2008; Belz et al., 2012; Heitmann et al., 2015; Simsek and Martinez, 2016). In terms of controlling gluten strength, NaCl works by screening charged amino acid residues on the gluten proteins to induce a greater amount of protein-protein aggregation, facilitated through hydrophobic interactions and then

disulphide bridging, resulting in a more ordered dough network. In the absence of salt, a less ordered network reducing its ability to abide water, which could result in increased dough stickiness and, thus, poor machinability (Stone et al., 2017). In addition, lower NaCl concentration may result in poorer dough handling properties as consequence of a weaker gluten network formed which cannot retain the gas produced. This can contribute to result in lower loaf volume and increased crumb hardness (Yovchev et al., 2017).

Flour. See Section 2.1.3

*Water***.** Water is the second most abundant ingredient in bread formulations, and impacts the overall characteristics of the dough and, consequently, final product (Mondal and Datta, 2008). It hydrates the gluten proteins, starch and other polysaccharides within the dough. Furthermore, it acts as a solvent for the other ingredients, a medium for biochemical and chemical reactions, being a determinant factor for product shelf life (e.g., medium where microorganisms grow) (Scanlon and Zghal, 2001; Giannou et al., 2003; Mondal and Datta, 2008; Simsek and Martinez, 2016).

*Yeast***.** Yeast (*Saccharomyces cerevisiae*) is an essential ingredient to dough, it is added as a leavening agent working to convert simple sugars, including sucrose and products of damaged starch (e.g. maltose, dextrose) into ethanol, carbon dioxide (CO_2), and energy (Delcour and Hoseney, 2010b). In addition, it can improve the rheological properties of dough (increase in elasticity), contribute to flavor, and the removal of phytic acid (Collado-Fernàndez, 2003a; Rezaei et al., 2019). The temperature plays an important role in yeast activity, where the activity increases with the temperature until an optimal range from 20-40 ºC (38 ºC highest gas production). With the further increase in temperature the activity progressively slows down until inactivation (~55 ºC). The presence of yeast can impact processing (i.e., machinability), shelf life, texture, taste, and flavor of the final product (Collado-Fernàndez, 2003b; Mucahit, 2012; Heitmann et al., 2015).

2.2.2 Non-essential ingredients in dough

Sugars. Sugars are important in the early stages of fermentation as an energy source for the yeast. Sugars at higher concentrations (>4% - based on Canadian Short Process) may be also added to increase gas production during fermentation, improve crust color, and/or sweeten the final product (Giannou et al., 2003).

Shortening or fat. Although lipids are lower in quantity within the flour than other components (proteins and starch), they can improve the functional properties and the final product (e.g., softness, moistness, flavor, and texture). Lipids can be originated from three main

sources including wheat flour, shortenings or margarine, and surfactants (Ohm and Chung, 2002; Giannou et al., 2003; Stauffer, 2007; Pareyt et al., 2011). Wheat flour contains ~2.0–2.5% total lipids, whose magnitude is dependent on the genetics, environmental, and milling process characteristics (Stauffer, 2007). In addition to wheat flour lipids, either shortening or margarine can be added to bread formulations in a range from 2% to 4% of flour weight. These lipids can influence mouthfeel, lubricity, flavor, dough handling, volume, gas retention, water absorption, and mixing time (Smith and Johansson, 2004; Goesaert et al., 2005; Stauffer, 2007; Aquino, 2012; Gerits et al., 2014a).

Emulsifiers. Emulsifiers, such as diacetyl tartaric esters of monodiglycerides (DATEM), ethoxylated monoglycerides (EMG), are ingredients containing both hydrophilic and hydrophobic ends. This characteristics affect dough strength and softens the crumb, by increased interactions with the proteins and starch (antistaling), respectively (Tebben et al., 2018). Furthermore, the addition of emulsifiers reduces the degree of starch swelling and solubilization during gelatinization, restricting starch polymer mobility and amylose leaching. (Gray and BeMiller, 2003; Gomez et al., 2004; Goesaert et al., 2005). According to Tebben et al. (2018), emulsifiers can also have a great effect on fermentation stability, improved dough elasticity, and higher tolerance for water absorption.

Dairy products. Dairy products include milk and whey containing lactose. The main role of dairy products is to promote browning, softer crust, and longer shelf-life (Collado-Fernàndez, 2003a).

Preservatives. Bakery products have a very short shelf life, where the quality is dependent on the time interval between baking and consumer's consumption. Bread freshness is determined by flavor, appearance and crispness of the crust, hardness of the crumb, and loaf volume. All these factors can be affected during storage which can be prevented and/or retarded by different physical methods (e.g., heat treatment, cold storage, and modified atmosphere storage) (Denkova et al., 2014). Preservatives might be added to breads with higher moisture (~40%) to reduce microbial and mold growth, resulting in longer shelf-life. The most commonly used preservatives are calcium propionate (E282), sorbic acid (E200), and vinegar (Collado-Fernàndez, 2003a).

2.2.3 Bread making process

Bread making consists of a number of steps including mixing or dough formation (i.e., mixing all the essential and non-essential ingredients), fermentation and baking (Pauly et al., 2013). This can be achieved using different bread making systems, such as straight-dough,

sponge-and-dough, liquid-sponge, and short-time bread making (Chorleywood) (Delcour and Hoseney, 2010b). The straight-dough is simple and most commonly used, where all the ingredients are mixed into a developed dough, where then it is allowed to ferment (~3 h), followed by molding (into pans), proofing, and baking (Tronsmo et al., 2003; Delcour and Hosetney, 2010b). Although each different system of bread making can vary slightly in each step (e.g. time), the main basic categories for bread making are dough formation (i.e., mixing), fermentation, and baking (Tebben et al., 2018).

Dough formation

Mixing is the process in bread making where all the ingredients, such as flour, water, yeast, salts, and additives, are mixed evenly through a mechanical process resulting in a homogeneous mass (Cauvain, 2015a). During this process, the gluten network is formed by hydration and the interaction of gliadin and glutenin and re-orientation of glutenin via S-S interchange forms the cohesive and viscoelastic properties of the dough (Collado-Fernàndez, 2003a; Weiser, 2003; Wrigley et al., 2006; Damodaran, 2007; Joye et al., 2009; Delcour and Hoseney, 2010b). In addition, during mixing, the lipids interact with the gluten proteins to help facilitate protein aggregation via hydrophobic interactions. The gluten-lipid interactions within the dough helps to improve dough handling, breadcrumb texture and flavor, along with shelf life (Demiralp et al., 2000; Giannou et al., 2003; Joye et al., 2009). The final step is the air uptake forming the nuclei of the gas cells. The time it takes to reach maximum dough strength during kneading is used as a measure of wheat quality for bread making—a longer time indicating better quality (Damodaran, 2007). During mixing, an optimally developed dough is formed having enough extensibility (i.e., to allow the dough to inflate with CO_2 production) to resist collapse, maintaining stability of the gas bubbles. However, over or under mixing of the dough are critical parameters that tend to result in undesired dough characteristics. When over mixed (i.e., after the optimal dough development) the dough is usually sticky, wet and weak, because the gluten network breaks down. In contrast, when under mixed the starch and proteins are not completely hydrated, resulting in poorer dough handling properties (Delcour and Hoseney, 2010b; MacRitchie, 2016).

Fermentation

Fermentation is an anaerobic process in breadmaking intermediated by yeast (*Saccharomyces cerevisiae*). First, the yeast is activated by oxygen then switchover to an anaerobic process. Fermentable sugars (e.g., free sugar from flour, sugars obtained by the action

of enzymes, or added as additives) are converted into carbon dioxide, ethanol, and aromatic compounds (Collado-Fernàndez, 2003b; Cauvain, 2015b). Thus, the pH is affected, dropping from ~6.0 to 5.0, as the carbon dioxide is produced and dissolved into water (Delcour and Hoseney, 2010b). The dough development is continued, becoming drier, less sticky, more elastic, and with improved gas retention (Weiser, 2003; Collado-Fernàndez, 2003a). Compounds that may be present in the dough (e.g., calcium propionate, acetic acid, and salt) can inhibit fermentation. Therefore, many factors may affect fermentation, such as flour strength (weak or strong), enzymatic activity of flour (amylases action), formulation (usually 1.5-2.0% of yeast), and yeast activity (Wieser, 2003).

Baking

After dough concludes the fermentation and proofing process, the baking step induces physical, physicochemical and biochemical changes to result in the desired product (Cauvin, 2015b). The bread dough expands over ~50% in volume (oven spring) in the first 5-10 min as a result the production of CO_2 until yeast is inactivated and the evaporation of water, CO_2, and ethanol (Wieser, 2003). In the oven, the heat is transferred to the dough through convection, conduction, and radiation, therefore different product temperatures are accomplished. For instance, the surface has a higher temperature and becomes drier (>100^{o}C) and the crumb softer with higher moisture (<100^{o}C) (Collado-Fernàndez, 2003b).

2.2.4 Bread quality

Even though many theories and concepts exist on good bread quality, certain quality standards are expected for individual bread varieties. Bread is directly influenced by the dough handling properties, especially, the gluten network strength to retain the carbon dioxide produced by the yeast (Joye et al., 2009). Thus, the dough requires a combination of strength, extensibility and tolerance, that depends mostly on flour quality, water absorption, and mixing conditions (Joye et al., 2009; Cauvin, 2012).

The HMW-GS subunits have the largest effect on bread making quality (Payne et al., 1984). On the other hand, the allelic variation of the LMW-GS could be associated with bread dough quality and extensibility, due to their ability to form intermolecular disulfide bonds with each other and with HMW-GS, directly affecting gluten polymer formation (Rasheed et al., 2002). In Canada, a relative high proportion of wheat varieties have the Glu-D1 5+10 allele and many have the Glu-A1 2* and the Glu-B1 7+9 subunits encoding HMW-GS (Békés et al.,

2006). Regarding the most frequent LMW-GS alleles, Canadian breeders have made extensive use of Glu-A3e, the "null allele", and many have the *Glu*-B3h (Fleitas et al., 2019).

Determining the rheological properties of the dough, such as the empirical rheological methods (i.e., farinograph, mixograph, and extensograph), and other rheology tests (i.e., creep-recovery and oscillatory tests) is crucial. Even though empirical methods are practical, easy to use and standardized, they are not sufficient to interpret the mechanical behavior of dough independent of the measurement device and its geometries. The correlations between these rheological characteristics and loaf quality are just valid on a very restricted range of flour properties (Bockstaele et al., 2008; Jekle, 2012). In addition, the final loaf can be evaluated by measuring the loaf volume and crumb texture properties of the baked loaves (MacRitchi, 2016). Crumb parameters include crumb firmness, cohesiveness, springiness and resilience, and secondary mechanical characteristics, as gumminess and chewiness (Sahli, 2015). In addition, crumb grain characteristics can be determine by digital image analysis (C-cell), focusing in parameters such as crumb brightness, mean cell area (mm^2), cell density ($cells/cm^2$; higher levels denote finer structure), cell to total area ratio (or void fraction, computed as the percentage of the total analyzed square occupied by detected cells), mean cell wall thickness (mm; calculated as the averaged mean intercellular distance of neighboring cells sampled) and crumb grain uniformity (Caballero et al. 2007).

Canadian Short Process – CSP

Originally, this method was developed to access the bread making quality of Canadian wheat flours to assess breeder lines submitted for cultivar registration and showing potential for the premium Canada Western Red Spring class (Grain Research Laboratory, 2016). The method is described by Preston et al. (1982) using commonly used additives in the baking industry (ascorbic acid, whey protein, malt, and shortening) and shorter fermentation time. However, in 2015, the Grain Research Laboratory developed lean no time (LNT) test bake method in substitution to CSP method. The main reason was to improve the discrimination of inherent dough strength (Grain Research Laboratory, 2016). In addition to being more discriminating, the LNT was easily adopted by other laboratories, due to its simplicity and relation to high throughput test baking conditions encountered in the evaluation of large numbers of breeder lines (Dupuis and Fu, 2017).

2.3 Flour additives for dough conditioning

Bread improvers can be incorporated to the formulation to overcome the deficiencies in bread making quality, such as oxidants, reductants, and enzymes (Tang et al., 2014). These exogenous components usually alter the gluten proteins functionality during the bread making process (Gomes-Ruffi et al., 2012; Joye et al., 2009). Even though chemical additives are largely used by the bread industry, certain synthetic oxidants (e.g., ADA) have been under studies and regulatory changes due to related health risks they may cause (Ye et al., 2011; Tebben et al., 2018). For this reason, the baking industry is investing in alternatives, such as enzymes since they function in a similar manner and are considered a processing aid enabling companies to keep them off the label (Caballero et al., 2007). In addition, there is an increasing demand for products with cleaner labels. Thus, products with less ingredients on the packages tend to be preferred by consumers (Cassiday, 2017).

2.3.1 Chemical oxidizers

Oxidizing agents include ascorbic acid, azodicarbonamide, potassium iodate and potassium bromate, and are widely used in the baking industry for their ability to modify dough properties (Sahi, 2014). Generally, oxidizing agents target the SH and S−S groups within the gluten to alter the strength of the gluten network and its resulting viscoelasticity. As result, the use of those substances can increase the dough development time and stability, lower the extensibility (increased strength), and alter the water absorption (Tebben et al., 2018).

Azodicarbonamide

Azodicarbonamide (ADA) is one of the fastest oxidants used as a dough improver in bread making for maturing flour commonly used in the United Stated and Canada (Joye et al., 2009, Ye et al., 2011). ADA is stable in dry flour, however, the reaction occurs within minutes after flour and water are mixed during dough processing, where ADA is reduced to biurea during the oxidation of sulfhydryl groups (Becalski et al., 2004; Noonan et al., 2008). In addition, semicarbazide (SEM) and urazole are also formed during the reaction (Figure 2.2). As result, a cohesive dry dough is formed that can tolerate high water absorption and can act to strengthen the dough to increase its resistance to extension. Furthermore, the mixing time is shortened and less energy input is required to mix the dough (Tsen, 1963; Wieser, 2003). In addition, as ADA is a fast acting oxidant it is known to strengthen the dough at optimum mixing time, however, it breakdown when overmixed resulting in the opposite effect (Miller and Hoseney, 1999).

In some studies, ADA presented to cause allergic reactions in those sensitive to other azo compounds or has been previously shown to heighten the allergic reaction of other ingredients in other foods (Ye et al., 2011). Recently, the additive has raised health concerns within the U.S. Food and Drug Administration (FDA, 2016) since the formation of SEM has been linked to the development of some forms of cancer (Bhagan et al., 2016; Kornbrust et al., 2012) and potential DNA damage induced by semicarbazide (SEM) (Hirakawa et al., 2003). In addition, semicarbazide-derived free radicals participate in DNA damage, which may be relevant to the carcinogenicity of semicarbazide. Other studies reported that oral administration of semicarbazide results in angiomas, angiosarcomas and lung cancer in mice (Toth, 2000). The possible formation of SEM is from thermal decomposition of biurea, which is formed during dough mixing and kneading (Noonan et al., 2008). Recently, ADA was under review within Canada and the United States, however it is already banned in the European Union, Australia and New Zealand, and Singapore (EFSA, 2005; Landau, 2014). The addition of ADA in Canada is limited by Health Canada (2012) to a maximum limit of 45 ppm (mg/kg).

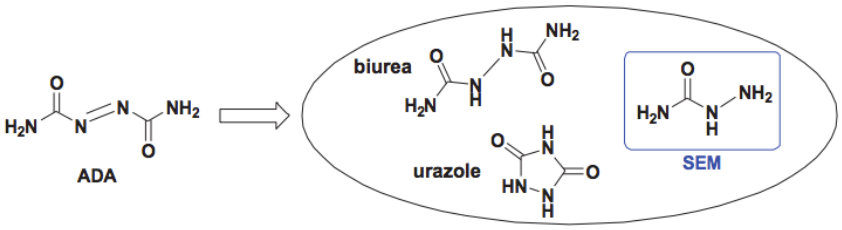

Figure 2.2. Thermal decomposition of azodicarbonamide (ADA) during baking, forming biurea, semicarbazide (SEM), and urazole (Bhagan et al., 2016).

Ascorbic Acid

Commonly known as Vitamin C, ascorbic acid is also used as a flour improver in the bread industry as an alternative to potassium bromate (Joye et al., 2009). Ascorbic acid itself is a reducing agent, however in the presence of oxygen and ascorbic acid oxidase, it is converted into the dehydro form, which then takes part in the SH/SS interchange oxidation reaction (Figure 2.3) (Nakamura and Kurata, 1997). Also, it can react with glutathione, which is known to negatively affect SH/SS reactions to result in weaker gluten network. As a result, its use typically leads to increased loaf volume and thinner crumb structure (Sahi, 2014; Delcour and Hoseney, 2010b). In addition, the use of ascorbic acid have effects on dough rheology characteristics, for instance, Miller and Hoseney (1999), presented increased elastic and

viscous moduli (G' and G") of two strong flours (Karl and Glenlea), indicating stronger dough pattern which can also explain higher bread loaf volume. According to Health Canada (2012), ascorbic acid can be added to the flour to a maximum limit of 200 ppm (mg/kg).

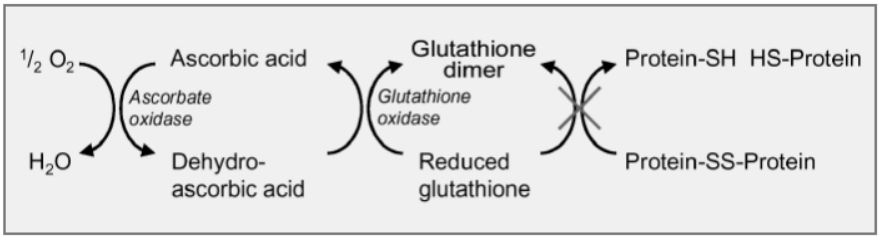

Figure 2.3. Ascorbic acid reaction in wheat dough (Popper et al., 2006).

2.3.2 Chemical Reductants

Reducing agents are a type of dough conditioner used to reduce mixing time and improve dough extensibility by breaking down SS bonds within the gluten network and converting them into SH groups, thus reducing the average molecular weight of glutenin protein aggregates (Wieser, 2003). The glutenin chains flexibility may be affected by the SH groups, resulting in the initiation of polymerization reactions (Lagrain et al., 2007).

L-cysteine

Some wheat flours have low deformation index limiting the CO_2 retention during the fermentation process, as result, lower loaf volume and porosity (Stoica et al., 2013). Thus, in order to relax the gluten increasing the extensibility, reducing agents such as L-cysteine can be done. L-cysteine (L-cys) is an accepted food additive, which is added to bread flour (up to 90 mg/kg, wheat flour basis) (Health Canada, 2012). This reducing agent is commonly used due to its action through reducing gluten proteins disulfide bonds, resulting in lower dough tenacity and elasticity, which reduces the mixing time of the dough and aids faster dough development (Figure 2.4) (Wieser, 2003; Majzoobi et al., 2011). Miller and Hoseney (1999) evaluated the dynamic rheological properties of dough with the addition of L-cysteine and other oxidants. L-cysteine, overall, decreased both storage and viscous modulus (G′ and G″, respectively) and increased tan δ, indicating a weaker dough characteristic. In agreement to this findings, Angioloni and Dalla Rosa (2007) also determined the dough rheological properties and mixing time with the addition of L-cysteine. As result, increased fluid-like characteristics to the dough, which had lower dough hardness, resistance to extension, and storage modulus (G′') and higher extensibility.

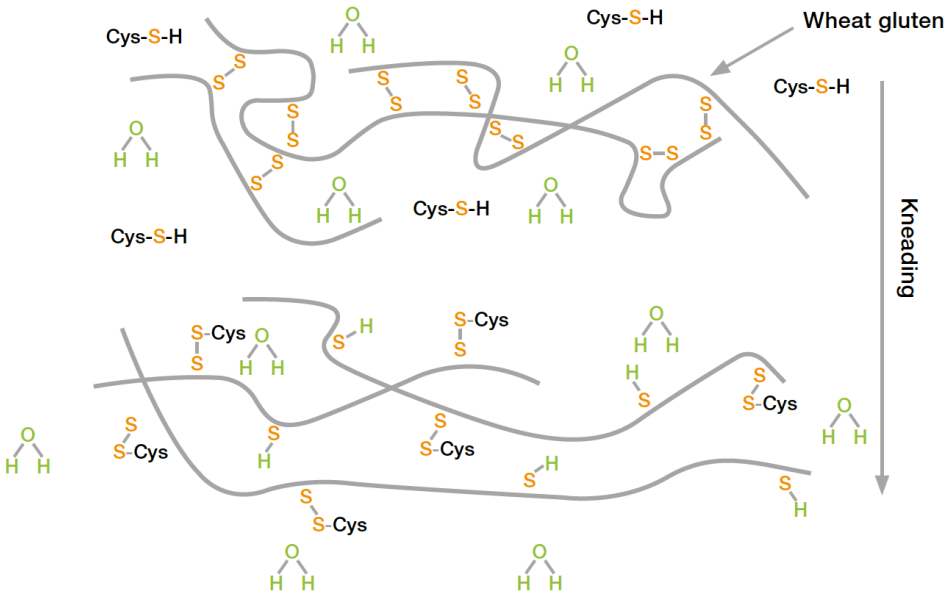

Figure 2.4. L-cysteine mechanism of action during dough mixing (WACKER, 2019).

2.3.3 Enzymes

Enzymes are a class of proteins that catalyze biochemical reactions by lowering the activation energy resulting in increased reaction rates and no change in reaction equilibrium (Sahi, 2014; Kornbrust et al., 2012). Enzymes can occur naturally (e.g., α-amylase) and/or be added during the process (e.g., glucose oxidase). They are classified into six main classes: [1] oxidoreductases (EC1), which catalyze oxidation/reduction reactions by transferring electrons (hydride ions or H atoms); [2] transferases (EC2), which transfers a functional group; [3] hydrolases (EC3), which catalyzes the hydrolysis of various bonds, transferring functional groups to water; [4] lyases (EC4), which cleaves various bonds by means other than hydrolysis and oxidation, adding groups to double bonds, or formation of double bonds by removal of groups; [5] isomerases (EC5), which catalyzes isomerization changes within a single molecule; and [6] ligases (EC6), which joins two molecules with a covalent bond (Nelson and Cox, 2005). In addition, because they are selective and specific, efficient and do not leave undesirable by-products, enzymes have become tools for the baking (Zhang et al., 2018). The global baking enzymes market is estimated to be US $607 million in 2020, expecting to reach almost US$ 1 billion by 2025, with a growth rate of 8.6% from 2020 and 2025 (Market Data Forecast, 2019). This increase may be a reflection of an ever-increasing demand for more natural products, thus

enzymes gained real importance as ingredient-aids, where they improve dough and bread quality by speeding reactions (Butt et al., 2008).

In the baking industry, the use of enzymes can be sourced from endogenous enzymes in flour, enzymes associated with the metabolic activity of the dominant microorganisms, and exogenous enzymes which are added in the dough (Miguel et al., 2013). The individual and combined use of a wide range of enzymes is increasing as bakeries attempt to optimize flour functionality, stabilize processing parameters, and improve dough quality (Kornbrust et al., 2012; Caballero et al., 2007). The use of enzymes can extend shelf life, improve dough fermentation, dough machinability and stability, increase loaf volume, develop finer and whiter crumb structure, and intensify crust color (Kuraishi et al., 2001; Goesaert et al., 2006; Caballero et al., 2007; Giannone et al., 2016). The most commonly used enzymes in baking are the oxidoreductases (e.g., glucose oxidase) and hydrolases (e.g. amylases, proteases, hemicelluloses and lipases) (Kornbrust et al., 2012).

2.3.3.1. Oxireductases

Oxireductases enzyme class is composed of enzymes that catalyze the exchange of electrons or redox equivalents between a donor (reductant) and an acceptor (oxidant) molecule (Kornbrust et al., 2012). They can be sub-classified in four different groups depending on their function, such as oxidases, peroxidases, oxygenases and dehydrogenases (Marcia et al., 2009).

Glucose Oxidase

Glucose oxidase (Gox) (EC 1.1.3.4) is a protein that is homodimeric and contains two similar polypeptide chain subunits (Raveendran et al., 2018). Even though many bacterial species are capable of producing Gox, fungi is usually considered for the industrial production. Thus, Gox can be sourced from various microorganisms, however, it is most commonly produced from *Aspergillus niger* and *Penicillium glaucum* (Kona et al., 2001; Raveendran et al., 2018). Gox catalyzes the oxidation of β-D-glucose to D-glucono-δ-lactone and hydrogen peroxide (H_2O_2) using molecular oxygen as an electron acceptor (Kona et al., 2001; Amiri et al., 2016).

In the baking industry, Gox catalyzes the formation of hydrogen peroxide (H_2O_2), which can oxidize the sulfhydryl groups forming of disulphide and non-disulfide crosslinks (Amiri et al., 2016). As result, it promotes the formation of disulfide bonds in the gluten network from the release of hydrogen peroxide from its catalytic reaction (Miguel et al., 2013). As result,

better dough machinability, improved gas retention, higher bread volume and finer crumb structure is obtained. The crosslinks are induced by coupling two cysteine residues within a protein matrix, as result an improved viscoelastic and structural properties, as well as better bread making performance (Tang et al., 2014). According to Bonet et al. (2006), Gox addition promoted an increase in dough stability when overmixing and modified the alveograph parameters. In other words, the addition resulted in a more resistant and less extensible dough, leading to loaf with greater specific volume and better shape. The same changes were also showed in other studies (Bonet et al., 2007; Decamps et al., 2012; Stoica, 2013). In another study, the addition of glucose oxidase resulted in a decrease of the resistance to extension of whole wheat dough to a level similar to that of white dough (Altınel & Ünal, 2017). In contrast, Rasiah et al. (2005) results show no significant difference in loaf volume between Gox treated bread and controls, as well as, no large differences in crumb structure were found. Altınel & Ünal, (2017) concluded that Gox exhibits different effects on dough and bread properties depending on type of flour and dosage of enzyme. Therefore, Gox may be considered as an alternative to the use of chemical oxidant agents in bread making as the mechanism by which hydrogen peroxide affects dough rheology appears to be similar to that of other oxidants (Miller and Hoseney, 1999; Stoica et al., 2009; Stoica, 2013).

2.3.3.2. Hydrolases

This family of enzymes catalyze the hydrolysis reactions, transferring functional groups to water (Nelson and Cox, 2005). They can be endo- and exogenous enzymes hydrolyzing the α-1,4 and ß-1,6 linkages in starch polymers, resulting in α-dextrins, maltose and glucose molecules (Rossell et al., 2001). These enzymes can be obtained from fungal, cereal or microbial sources. The main difference between those is the thermo stability, which can directly impact their function and application.

Xylanase

Xylan and glucomannan are the two main types of hemicelluloses, which are considered the most abundant heteropolymers in the nature (Haddar et al., 2012). Xylan is a group of hemicelluloses with a linear backbone of β-1, 4-linked D-xylopyranose residues with acetyl, arabinosyl, glucuronosyl, mannosyl, and uronosyl groups in the side chains (Verma et al., 2013). Due to the high water absorption of xylans, they have an important role in bread making quality and interaction with gluten (Butt et al., 2008). This high water absorption can be related to the arabinoxylans (AX) which constitutes the major non-starch polysaccharides in wheat

flour and can absorb up to four times its weight in water. In addition, AX can be divided in water-extractable and water-unextractable AX (Xu et al., 2016).

Xylanase (EC 3.2.1.8) are genetically single chain glycoproteins, ranging from 6–80 kDa (Butt et al., 2007). These enzymes are part of the hydrolase group, which consist in various endo- and exogenous enzymes that hydrolyze the α-1,4 and ß-1,6 linkages in starch polymers, resulting in α-dextrins, maltose, and glucose molecules (Rossell et al., 2001). It can be obtained from filamentous fungi or bacteria (including Bacillus strains), with main the differences being the thermo stability, which can directly impact their function and application (Haddar et al., 2012; Verma et al., 2013; Ghosha et al., 2017). Filamentous fungi tend to be more attractive as their enzymes are secreted into the medium and have higher producing levels than yeasts and bacteria (Terrasan et al., 2016). Among filamentous fungi, *Trichoderma* and *Aspergillus* are within the most efficient producers of xylanolytic enzymes (Romanowska et al., 2006). Usually, xylanases are active at temperatures from 40-60 °C and in acidic or neutral pH (between 4.5 and 6.5) (Butt et al., 2007). In contrast, only few xylanases were reported to be active and stable at alkaline pH and high temperature (Dhillon et al., 2000; Terrasan et al., 2016). The increasing interest in xylanases is due to their diverse range of application from pulping and bleaching processes to bioethanol production process (Verma et al., 2013). In Europe, it is a common practice the addition of xylanase to bread formulation as a means to improve its quality and crumb structure (Xu et al., 2016).

In bread making, xylanases degrade arabinoxylans and other xylans reducing the viscosity of the wheat flour dough, thus contributing to improve dough handling properties (Ghosha et al., 2017). Due to arabinoxylan degradation, the water is redistributed from the flour pentosans to gluten, resulting in an increase in bread volume and crumb quality, and an antistaling effect (Grossmann et al., 2016). This effect can be enhanced if amylases are used in combination with xylanases (Leon et al. 2002; Pastor et al., 2007). Verjans et al., (2010) observed an improve in bread loaf volume xylanases by 24% under the conditions of the process with the addition of two different types of xylanase (from *Aureobasidium pullulans* and *Bacillus subtilis*). Same trend was found by Xu et al. (2016), analyzing Chinese steamed bread where the addition of xylanase from *Streptomyces sp.* FA1 led to a greater increase in the specific volume in comparison to control and commercial xylanase under optimal conditions. This increase may be explained by the hydrolysis of water-insoluble arabinoxylan and released smaller oligosaccharides, which absorb far less water (Romanowska et al., 2006). Passarinho et al. (2019) expressed mutated and non-mutated xylanases in *E. coli* and evaluated their effect on sponge bread dough. A significant volume increase was presented when compared to the

control with the use of xylanase. The positive effect on dough was attributed to the water molecules redistribution within the system after arabinoxylan hydrolyses, as consequence more intermolecular interactions between the gluten proteins which resulted in a more extensible dough. In addition to that, simple sugars were released and boosted the substrate for the yeast accelerating the CO_2 release, which could also impart in the dough volume (Passarinho et al., 2019).

3. THE INTER-RELATIONSHIPS BETWEEN WHEAT QUALITY, COMPOSITION AND DOUGH RHEOLOGY FOR A RANGE OF CANADIAN WESTERN WHEAT CULTIVARS

3.1. Abstract

The overall goal of this research was to understand the inter-relationships between wheat quality, grain and flour composition and dough rheology for a range of commercially grown Canadian wheat (*Triticum aestivum* L.) cultivars (×25) within different wheat market classes. Cultivar-type varied in proximate composition which directly impacted dough handling parameters. Micro-doughLAB absorption was positively correlated with protein concentration, grain hardness, wet gluten and dry gluten content, and was negatively correlated with the gluten performance index. Strong and significant correlation was found between gluten properties, flour composition, and dough strength measurements. Protein and gluten properties in particular, significantly impacted dough strength measurements. Cultivars displaying stronger gluten strengths may result in dough with better dough handling properties. The study of 25 commercially grown Western Canadian wheat cultivars with a range of gluten strength, and their relation to their glutenin and gliadin subunit composition, and dough handling properties.

Significance and novelty: The evaluation of a vast set of 25 cultivars commercially grown in Western Canada within different wheat classes. Analyzed their relation to the new wheat classification made based on performance quality parameters and market needs. Evaluation of rheological parameters using rheometer method.

3.2. Introduction

Wheat is one of the most important crop in the world, since it can be grown under diverse climatic zones, elevation, or edaphic factors, it is considered to be one of the main plant based food that provides calories and proteins to the global population (Jones, et al., 2015). In Canada, wheat is the major cereal crop predominantly grown in the three Prairie Provinces, Manitoba, Saskatchewan, and Alberta (SaskWheat, 2019). Canadian wheat has good grain quality that makes it a highly sought after commodity in the international market place (SaskWheat, 2019). Wheat grain and flour quality are dependent on wheat genotype,

environment, agronomic practices, including soil fertilization, harvesting, and the milling process. Some cultivars are more influenced by growth conditions than others; thus, interactions between genotype and environmental factors significantly influence both, grain quality and flour functionality (Wrigley, 2009).

Wheat is unique as it is the only cereal grain whose flour when mixed with water forms a three-dimensional viscoelastic dough (Bockstaele, et al., 2008). Therefore, the wheat flour quality depends on the protein content and composition (gliadins and glutenins), responsible for gluten formation, directly influencing the end-product characteristics (e.g., bread crumb structure and loaf volume) (Collado-Fernández, 2003; Caballero, et al., 2007; Joye, et al., 2009). Gluten is formed by two different proteins, glutenin and gliadins. The glutenins contribute 30-40% of the flour protein and are divided in low- and high-molecular-weight fractions (LMW-GS and HMW-GS). The HMW-GS subunits have the most effect on breadmaking quality (Payne, et al., 1984). Therefore, the glutenin polymer structure, size distribution, and subunit composition, and the gliadin/glutenin ratio strongly affect gluten quality and, as consequence, the bread-making potential of wheat flour (Joye et al., 2009). The allelic variation of the LMW-GS has also been associated with dough quality and extensibility, due to their ability to form intermolecular disulfide bonds with each other and with HMW-GS, directly affecting gluten polymer formation (Rasheed et al., 2002). In Canada, high proportion of wheat varieties have the *Glu-D1* 5+10 pair of subunits and many have the *Glu-A1* 2* and the *Glu-B1* 7+9 subunits encoding HMW-GS (Békés et al., 2007). The LMW-GS *Glu-A3e* (the "null allele"), which is usually associated with inferior quality parameters, has been the most frequent allele for decades (Fleitas et al., 2019); however, Canadian breeders have made extensive use of other alleles with positive influence on dough strength. Thus, several cultivars have the LMW-GS *Glu-B3h* or the *Glu-B3g* combined with HMW-GS alleles with excessive strength such as the *Glu-B1* 7oe+8 subunit pair, (over-expressed subunit 7) an important contributor to strength (Fleitas, et al., 2019).

Furthermore, the characterization of the wheat grain and derived flour, and the flour rheological properties is fundamental to predict the processing behaviour and, consequently, the quality of the end-products (Song and Zheng, 2007). Glutenin and gliadin subunit composition and concentration directly influence gluten strength and, thus, impact dough rheology properties. The latter can be determined by empirical and fundamental rheological tests, such as farinograph, mixograph and extensograph, and rheometry (e.g., small amplitude oscillatory shear and creep recovery), respectively (Song and Zheng, 2007; Jekle and Becker, 2011).

Wheat classification into grades is essential for pricing and trading purposes, as it allows customers to define their requirements according to specific end products for consistency and performance (Cracknell and Williams, 2004). Stability of quality characteristics is an important requirement in the baking industry. The major factors used to distinguish wheat genotypes in the market are grain hardness (hard or soft), bran colour (red or white), growth habit (winter or spring), and grain protein content and composition (gluten strength) (Carson and Edwards, 2009). In Canada, the largest and most exported wheat market class is the Canada Western Red Spring (CWRS) typically used for the production of high-volume pan and crusty bread. CWRS cultivars have excellent milling quality, and the flour is characterized by high farinograph water absorption (between 64 to 70%), and well-balanced gluten strength suitable for both, straight dough (i.e., all ingredients (dry and liquid) are placed in the mixer and the dough is then mixed to full development) and long-fermentation sponge and dough baking process (i.e., bulk fermentation period is used) (Carson and Edwards, 2009).

The Canadian wheat market class system continues to evolve over time to handle changes in the end-use quality types demanded by both the domestic and international industries (McCallum and DePauw 2008). In early 2015, the Canadian Grain Commission (GGC) proposed a new wheat classification system to modernize the process. The aim was to remove weak gluten cultivars from the CWRS and Canada Prairie Spring Red (CPSR) segregations to ensure class quality, consistency and end-use performance. CGC also implemented two new wheat classes, Canada Northern Hard Red (CNHR) and Canada Western Special Purpose (CWSP) so that segregations still exist for the varieties now excluded from the CWRS and CPSR classifications (CGC, 2018).

The goals of this research were (*i*) to evaluate the wheat flour quality of a range of commercially grown Western Canadian wheat cultivars (×25) selected to represent a range of gluten strengths based on historical data from different classes of Western Canadian wheat, including the new classes CNHR and CWSP and to (*ii*) to investigate the inter-relationships among wheat quality, flour composition and dough rheology of those cultivars.

3.3. Materials and methods

3.3.1. Plant materials and experimental design

Twenty-five wheat (*Triticum aestivum* L.) cultivars (Table 1) were selected to cover a range of wheat classes and dough strength (largely based on farinograph data from previous studies) with greater emphasis placed on CWRS as this market class contained four out of the five most grown cultivars in 2016 crop-year in Western Canada (CIGI, 2016). Accordingly

these four cultivars were included in this study (AAC Brandon, Stettler, CDC Utmost, and Carberry).

The grain was harvested in the 2016 year from a Randomized Complete Block Design (n = 3) field trial at the Kernen Crop Research Farm (52.158; -106.524; altitude 457 m), University of Saskatchewan (Saskatoon, SK, Canada). Plots consisted of five 3.7 m long rows spaced 20 cm apart, in a Sutherland clay, clay-loam soil. Seed placed fertilizer (28-23-0; N-P-K used as monoammonium phosphate, $NH_4H_2PO_4$) was applied at a rate of approximately 50 $kg.ha^{-1}$. All experiments were established on fallow land using a seeding rate of 300 $seeds.m^{-2}$. Meteorological conditions during the crop season (May to September) were obtained from the NASA Prediction of Worldwide Energy Resource (POWER).

Table 3.1. Western Canadian wheat cultivars per wheat class

Wheat Class	Cultivar	Characteristics	End-uses
Canada Western Red Spring (CWRS)	AAC Redwater, AAC Brandon, CDC Utmost, CDC Stanley, CDC Plentiful, CDC Titanium, Carberry, Glenn, Parata, Roblin, Shaw VB, and Stettler	- Hard red spring wheat - Superior milling and baking quality - 3 milling grades - Various guaranteed protein levels	- High volume pan bread - Alone or in blends with other wheat for hearth bread, steamed bread, noodles, flat bread, common wheat pasta
Canada Northern Hard Red (CNHR)*	Harvest, Lillian, McKenzie, Pembina, and Unity VB	- Red spring wheat - Medium to hard kernels - Very good milling quality - Medium gluten strength - 3 milling grades	- Hearth breads, flat breads, steamed breads, noodles
Canada Western Hard White Spring (CWHWS)	AAC Iceberg, CDC Whitewood, and Whitehawk	- Hard white spring wheat - Superior milling quality producing flour with excellent color - 3 milling grades	- Bread and noodle production
Canada Western Special Purpose (CWSP)*	Pasteur, CDC Kinley	- Generally, not appropriate for milling - Usually high starch and low protein content	- Most suitable ethanol product or animal feed
Canada Prairie Spring Red (CPSR)	5702PR, CDC Terrain	- Red spring wheat - Medium hard kernels - Medium dough strength - 2 milling grades	- Flat breads, steamed breads, noodles, hearth breads
Canada Western Extra Strong (CWES)	CDC Walrus	- Hard red spring wheat - Extra strong gluten - 2 milling grades	- Ideal for blending - Specialty products that need high gluten strength

*As of August, 2018 (CGC, 2019b)

3.3.2. Flour preparation

Grain (~5 g) for each cultivar was ground in a Thomas–Wiley laboratory grinder (model 4, Arthur H. Thomas Co.: Philadelphia, PA, USA). The grain mealseed was used to determine the moisture content following the AACCI Approved Method 44-15.02. From the determined moisture, approximately, 500 g of seed from each cultivar was tempered to 14.5% for ~18 h and, then, milled into flour on a Barbender Quadrumat Senior Experimental Mill (Brabender: South Hackensack, NJ, USA), as described (Jeffers and Rubenthaler, 1977) at the Grains Innovation Laboratory, University of Saskatchewan. For proximate analyses data was collected in duplicate on three biological replicates and presented as the mean ± standard deviation.

3.3.3. Grain and flour quality

The grain hardness index (HI) was determined using the Single Kernel Characterisation System, SKCS4100 (Perten Instruments, Springfield, IL, USA) using AACCI Approved Method 55-31.01. Grain and flour protein content (% N × 5.7) was determined using a combustion method (LECO Model FP-528, LECO Instruments Crop., St Joseph, USA) following AACCI Approved Method 4630.01. Flour moisture (%), ash content (%) and falling number (s) of all flours were measured according to AACCI Approved Methods 4415.02, 0803.01, and 56-81.03 respectively. Crude lipid content (%) was measured using an ANKOM Extraction System XT-15 (ANKOM Technology, NY, USA) following AOCS Standard Procedure Am 5-04 (AOCS, 2005). The damaged starch content in the flours was determined with a Megazyme assay kit (Megazyme International, Bray, Ireland) following the AACCI Approved Method 76-31.01. Gluten properties (gluten index, wet/dry gluten content) were assessed using the Glutomatic System (Perten Instruments, Springfield, IL, USA), according to AACCI Approved Method 38-12.02. The solvent retention capacity (SRC) test was performed based on the AACCI Approved Method 56-11.02, with modifications (Kweon, et al., 2011). SRC uses four solvents, including 5% lactic acid, 50% sucrose, 5% sodium carbonate, and distilled water as a means of predicting their contribution to the flour's overall quality. The test is largely applied to early generation screen, which requires test methods that need a small amount of sample and quick performing time (Xiao et al., 2006).

3.3.4. Gluten proteins extraction and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Gluten protein fractions (glutenins and gliadins) were sequentially extracted from grain meal using the method described by Singh, et al., (1991). The grain meal (20 mg) extraction was performed with 1 mL of propan-1-ol (50% v/v) at 65 ºC for 30 min with constant mixing using a Thermomixer (Eppendorf R, 1,400 rpm). The extraction mixture was centrifuged at 10,000 rpm for 2 min to separate the pellet containing the glutenins and the supernatant with the gliadins. Then, the supernatant was air dried overnight to concentrate the gliadins. The pellet was washed with 0.5 ml of propan-1-ol (50% v/v), centrifuged for 5 min and all the liquid was removed. The residue (pellet containing glutenins) was extracted with propan-1-ol (50% v/v), 0.08 M Tris-HCl, sodium dodecyl sulfate (SDS, 2% w/v), pH 8.0 and dithiothreitol (1.5% w/v) at 65 ºC with constant mixing for 30 min using a Thermomixer (EppendorfR , 1,400 rpm). The extraction mixture was centrifuged at 10,000 rpm for 2 min the glutenin peptides were treated with propan-1-ol (50% v/v), 0.08 M Tris-HCl, SDS (2% w/v), pH 8.0 and 4-vinyl pyridine (1.4% v/v) to alkylate the sulfhydryl (SH) groups, inhibit bonds from reforming. Both, the gliadin and glutenin peptides were denatured with SDS in the gel loading buffer. The denatured peptides were separated using denaturing polyacrylamide (15% w/v) gel electrophoresis (constant current 12.5 mA; 20 h; temperature 15 ºC). After electrophoresis, the separated polypeptides were visualized by staining with Coomassie blue (0.01% w/v). The nomenclature proposed by Payne and Lawrence (1983) was used for HMW-GS, whereas Gupta and Shepherd (1990), Jackson et al. (1996), Branlard, et al., (2003). and Appelbee et al. (2009) were used for both, LMW-GS and ω-gliadins. Gliadins were used as indicators of LMW-GS based on the linkage between LMW-GS and gliadins since gliadin composition can be screened more readily than specific LMW-GS. The glutenin and gliadin polypeptide analyses were done using grain from three biological replicates.

3.3.5. Micro-doughLAB and mixograph

The water absorption (ABS, %), dough development time (DDT, min), stability (STA, min) and mixing tolerance index (MTI) were measured using a Newport Micro-doughLAB mixer (Perten Instruments, Springfield, IL, USA) and calculated using doughLAB for Windows (DLW) software version 1.0.0.56. In brief, 4 g of flour from each cultivar was weighed based on a 14% m.b. (moisture basis) and mixed at a constant speed of 63 rpm. The amount of water added was determined as the water needed to achieve a dough consistency of

500 BU (Brabender Unit). Dough mixing properties were also measured using the Mixograph (TMCO National Mgf, Lincoln, NE), according to AACCI Approved Method 54-40.02.

3.3.6. Oscillatory shear rheometry and creep compliance

Each dough used for rheology was prepared following the AACCI Approved Method 54-40.02 in a 10 g Mixograph (TMCO National Mfg., Lincoln, NE). The formulation used was based on the basic dough ingredients, such as flour (weight on a 14% m.b.), water (weight based on micro-doughLAB absorption), and NaCl (2.0 % by weight). Each sample was mixed to peak dough development and allowed to rest for 60 min before being analyzed by the rheological testing. The small amplitude shear rheometry was performed based on the method of Jekle and Becker (2011) using an AR-2000 rheometer (TA Instruments, New Castle, DE, USA) with a 40 mm parallel plate fixture. Parameters such as the dynamic storage (G′, Pa), loss (G″, Pa), complex (|G*|, Pa), and loss tangent were determined as a function of frequency (0.1-100 Hz) at a constant amplitude strain of 0.1%. Values at a frequency of 1 Hz were arbitrarily selected for comparative purposes between cultivars. After the applied stress was removed, the relaxation of the dough, i.e. creep recovery, was performed on the same dough sample at a constant shear stress (τ_0 = 250 Pa) for 180 s. After this applied stress, stress was removed, and the relaxation of the dough was observed for 360 s. The maximum creep compliance (J_{max}, mPa^{-1}) and the relative elasticity (J_{el}, unitless) were calculated based on the method instructions. All oscillatory rheology measurements were made within the linear viscoelastic regime; however, the creep compliance was not. Data was collected in duplicate on each of the biological triplicate samples and presented as the mean ± one standard deviation (n = 3).

3.3.7. Statistics

All the determinations were made in duplicate (technical replicates) on each of the three biological replicates. The result is presented as mean ± SE (n = 3). A one-way analysis of variance (ANOVA) analysis with a Tukey Post-Hoc test was performed to determine differences among the 25 cultivars. In addition, Pearson correlations were conducted relating mixograph, microdough lab, and rheology data to composition. Principal component analysis (PCA) was used to better describe cultivar differences in each of composition/quality, mixograph, microdoughLAB, and rheology data. All statistical analysis were performed using SPSS Grad Pack v24 software (IBM, New York, USA).

3.4. Results and discussion

3.4.1. Allelic diversity of HMW-GS and LMW-GS

The allelic composition of HMW-GS and LMW-GS loci in each of the 25 wheat varieties showed a total of 6 HMW-GS alleles with 2, 3 and 1 alleles identified at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively (Table 3.2). Based on their mobility on SDS-PAGE (Figure A3.1 Appendix), all genotypes presented either the *Glu-A1* 1 and 2* subunits with none of the lines possessing the "null" allele. The subunit *Glu-A1* 2* was predominant, since 19 cultivars (76%) carried this subunit with the *Glu-A1* 1 subunit being present in the remaining 24% of genotypes studied (Table 3.2). The three HMW-GS *Glu-B1* subunit pairs present were 7oe+8, 7+9 and 14+15 with a clear prevalence of the first two. Finally, the HMW-GS associated with *Glu-D1* did not show diversity as all the genotypes carried the 5+10 subunit pair (Table 3.2). A high proportion of Canadian wheats have the *Glu-A1* 2*, the *Glu-D1* 5+10 subunits, while *Glu-B1* 7+9 and 7oe+8 are relatively evenly distributed among genotypes (Békés et al., 2007; Bushuk, 1998). However, a recent study revealed that the cultivars released after 1975 had a higher proportion the *Glu-B1* 7oe+8 (over-expressed subunit 7, an important contributor to strength) (Fleitas et al., 2019). Conversely, the *Glu-B1* 14+15 (only present in cultivar Pasteur), imparts negative effects on processing and end-use quality. For instance, Vancini et al. (2018) found that accessions with the *Glu-B1* 7oe+8 bands presented the highest gluten strength, elasticity index, and farinograph stability, differing from the pair of subunits 14+15 and 6+8 which showed the lowest values. It is well-known that *Glu-D1* 5+10 subunits is related to strong dough likely due to an additional cysteine residue present in subunit 5 which increases the formation of disulphide bonds resulting in glutenin polymers of higher molecular weight (Ikeda, et al., 2007). In previous studies, the 5+10 subunit pair was found to be associated with desirable breadmaking quality (Payne, et al., 1987; Patil, et al., 2015).

A total of 12 LMW-GS alleles were detected among the set of 25 wheat cultivars studied with four alleles identified at every locus (*Glu-A3*, *Glu-B3* and *Glu-D3*, Table 3.2). In this study, after the Glu-A3e allele, Glu-A3f was the next most common allele followed by Glu-A3d and Glu-A3g (52%, 32%, 8% and 8%, respectively). For alleles encoded by *Glu-B3*, *Glu-B3h* was the most prevalent at 52% while 40% had the *Glu-B3g* allele. *Glu-B3i* and *Glu- B3b'* were each only present in a single cultivar, CDC Terrain and CDC Titanium respectively. With regards to the *Glu-D3* locus, a total of four alleles were found in all genotypes (Table 3.2). *Glu-D3c* prevailed in 56% of the genotypes followed by the *Glu-D3a* allele in 32%. The *Glu-D3b* was detected in only two cultivars (Roblin and AAC Redwater) while the allele *Glu-D3e* was only present in the cv. Pasteur.

Table 3.2. Allelic composition at loci *Glu-A1*, *Glu-B1*, *Glu-D1* (encoding HMW-GS), *Glu-A3*, *Glu-B3*, *Glu-D3* (LMW-GS) and *Gli-B1* (ω-gliadins) for twenty-five Canadian cultivars.

Wheat Class*	Cultivar	Grain Protein**	HMW			LMW			ω-Gliadins
		(%)	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	*Gli-B1*
CNHR	Harvest	14.3	2*	7+9	5+10	f	h	c	d
CNHR	Pembina	13.7	2*	7+9	5+10	f	g	c	f
CNHR	McKenzie	13.6	2*	7+9	5+10	e	g	c	f
CNHR	Unity VB	13.7	2*	7+9	5+10	e	g	c	f
CNHR	Lillian	15.4	2*	7oe+8	5+10	e	h	c	d
CPSR	CDC Terrain	13.1	1	7oe+8	5+10	e	i	c	m
CPSR	5702PR	12.9	2*	7oe+8	5+10	g	g	c	f
CWES	CDC Walrus	13.8	2*	7oe+8	5+10	g	g	a	f
CWHWS	Whitehawk	13.9	1	7oe+8	5+10	e	g	c	f
CWHWS	AAC Iceberg	13.9	2*	7+9	5+10	f	h	a	d
CWHWS	CDC Whitewood	13.9	2*	7+9	5+10	f	g	a	f
CWRS	Roblin	15.2	2*	7oe+8	5+10	f	h	b	d
CWRS	Glenn	14.4	2*	7+9	5+10	d	h	c	d
CWRS	Carberry	14.6	2*	7+9	5+10	d	h	a	d
CWRS	CDC Utmost	14.2	1	7oe+8	5+10	e	h	a	d
CWRS	CDC Stanley	13.8	2*	7+9	5+10	e	g	c	f
CWRS	CDC Plentiful	14.6	1	7oe+8	5+10	e	h	c	d
CWRS	CDC Titanium	15.0	1	7oe+8	5+10	e	b’	a	b
CWRS	Shaw VB	14.2	2*	7oe+8	5+10	f	h	c	d
CWRS	Stettler	14.3	2*	7+9	5+10	e	h	a	d
CWRS	AAC Redwater	14.5	2*	7oe+8	5+10	f	h	b	d
CWRS	AAC Brandon	13.8	2*	7+9	5+10	e	h	c	d
CWRS	Parata	14.6	2*	7+9	5+10	f	h	a	d
CWSP	CDC Kinley	14.1	2*	7+9	5+10	e	g	c	f
CWSP	Pasteur	11.7	1	14+15	5+10	e	g	e	f

Abbreviations: Canada Western Red Spring (CWRS); Canada Northern Hard Red (CNHR); Canada Western Hard White Spring (CWHWS); Canada Western Special Purpose (CWSP); Canada Prairie Spring Red (CPSR); Canada Western Extra Strong (CWES)

* As of August, 2018 (CGC, 2019b).

The LMW-GS *Glu-A3* and *Glu-B3* alleles are known to play a major role in determining differences in processing qualities among the three *Glu-3* loci, while *Glu-D3* alleles play minor roles in determining quality variation in bread wheat. A prior study of 37 Canadian wheat genotypes revealed that a high proportion of lines (91%) released between 1860 and 1935 possessed the *Glu-A3e* allele but its prevalence was reduced to 58% in varieties released between 1935 and 2007 (Fleitas et al., 2019). The *Glu-A3e* allele is usually associated with negative effects on dough properties (Ikeda et al., 2007; Bonafede et al., 2016), although in some cases, the contrary has been true. For instance, Zheng et al. (2009) reported that the *Glu-A3e* allele was associated with better dough-mixing properties compared to other alleles. In the present study, after the *Glu-A3e* allele, the *Glu-A3f* was the next most common allele followed by *Glu-A3d* and *Glu-A3g* (52%, 32%, 8% and 8%, respectively). In our set, cultivars Glenn and Carberry from the CWRS carried the *Glu-A3d* allele which has been reported to have positive effects on gluten strength and dough properties (Branlard et al., 2003; Ikeda et al., 2007). Cultivars 5702PR (CPRS) and CDC Walrus (CWES) had the *Glu-A3g* usually found in Canadian extra-strong hard wheats such as cv. Glenlea. The *Glu-A3g* allele has been associated with increased gluten strength, and Funatsuky et al. (2007) reported that combined with the HMW-GS *Glu-D1* 5+10 subunits contributed to extra strong dough properties.

Negative effects of null alleles such as *Glu-A3e* and *Glu-B3j* might be compensated with excessive strength of the HMW-GS. However, a given allele may not necessarily show its positive effect in all genetic backgrounds since glutenin subunit loci interact with each other in the manifestation of dough strength. In Canada, several cultivars have the LMW-GS *Glu-B3h* or the *Glu-B3g* alleles combined with HMW-GS with excessive strength such as the *Glu-B1* 7oe+8, (over-expressed subunit 7) an important contributor to strength (Békés et al., 2007). The *Glu-B1* 7oe+8 subunits, originally common in South American wheats (Gianibelli et al., 2002; Békés et al., 2007), it has increased over the years in Canadian cultivars reflecting selection preference during the breeding process.

On the other hand, Zhang et al (2012), studying the composition and functional analysis of LMW-GS alleles with near-isogenic lines of bread wheat concluded that *Glu-D3* alleles played minor roles in determining quality variation. In the particular case of the *Glu-D3*, the identification difficulty and the use of different nomenclatures among laboratories has resulted in contradictory reports. The literature available regarding the effect of the *Glu-D3* alleles on breadmaking quality is scarce and further investigations are needed. Cultivar Pasteur (carrying the *Glu-D3e* allele) comes originally from the Netherlands and is valued for its high yield potential but is not broadly accepted as a milling wheat in Canada. Cultivar Orca, another

cultivar from the Netherlands, also carries the *Glu-D3e* allele (Gupta and Shepherd, 1990; Békés et al., 2015) and it is considered to be a soft-grained wheat with poor baking characteristics (Doekes and Wennekes, 1982).

3.4.2. Flour quality and composition

Anually, new wheat cultivars emerge from Canadian bread trials to be incorporated into the CWRS wheat class, which is marketing class of premium wheat (Dupuis and Fu, 2017). In the recent years, Carberry and Glenn cultivars have been used as cultivars checks in variety registration tests for dough strength and wheat quality for the CWRS wheat class (CGC, 2015). Carberry is at the lower end of acceptable dough strength, whereas Glenn sets the upper limit (Agriculture and Agri-Food Canada, 2019). Therefore, these cultivars can be considered as benchmarks to classify a cultivar as having weak, intermediate, or strong gluten strength characteristics and dough handling. The cultivars from CWRS that would not fall within the range (i.e., between Carberry and Glenn) were re-classified to CNHR, due to the concerns around the weak gluten strength and, thus, breadmaking performance. From the 25 cultivars set analyzed, five were re-classified from CWRS into CNHR (i.e., Harvest, Lillian, McKenzie, Pembina, and Unity VB). According to the results presented, CWRS cultivars had higher protein content (13.6%), wet gluten (39.4%), gluten index (82.2%), SRC lactic acid (152.8%), gluten performance index (0.83), stability (6.8 min) and dough development time (4.7 cm), when compared to CNHR (13.2%, 39.0%, 78.8%, 144.5%, 0.79, 5.3 min, and 3.5 min, respectively). All these parameters are related to dough and gluten strength not being suitable for CWRS class. Therefore, it sustains the idea of moving those cultivars to CNHR known to characterize a medium-low gluten and, consequently, dough strength.

Proximate analyses revealed that the overall average protein content, ash, lipid and damaged starch were found to be 13.2% (ranging from 10.5% - Pasteur to 14.8% - Roblin), 0.38% (ranging from 0.34 - CDC Whitewood to 0.42% - Lillian), 0.7% (no significant difference among cultivars), and 4.6% (ranging from 3.6 - CDC Stanley to 5.6% - Glenn), respectively (Table 3.3). The proximate composition of the wheat flour was similar to results reported by the CGC (2016).

Table 3.3. Proximate composition for twenty five wheat cultivars. Data represents the mean ± standard deviation from 3 biological replications (n = 3).

Cultivar	Wheat Class	Protein (%)	Ash (%)	Lipids (%)	Damaged Starch (%)
Harvest	CNHR	13.5 ± 1.1^{bcde}	0.41 ± 0.01^{abc}	0.8 ± 0.4^{a}	5.36 ± 0.22^{ab}
Lillian	CNHR	14.4 ± 0.5^{de}	0.42 ± 0.01^{bc}	0.8 ± 0.1^{a}	4.83 ± 0.93^{ab}
McKenzie	CNHR	12.8 ± 0.5^{bcd}	0.38 ± 0.02^{abc}	0.7 ± 0.5^{a}	5.59 ± 0.14^{b}
Pembina	CNHR	12.7 ± 0.4^{bcd}	0.39 ± 0.03^{abc}	0.8 ± 0.4^{a}	4.16 ± 0.24^{ab}
Unity VB	CNHR	12.4 ± 0.8^{abc}	0.37 ± 0.02^{abc}	0.7 ± 0.3^{a}	5.31 ± 0.29^{ab}
5702PR	CPSR	11.8 ± 0.7^{ab}	0.36 ± 0.02^{abc}	0.8 ± 0.1^{a}	4.33 ± 0.43^{ab}
CDC Terrain	CPSR	12.3 ± 0.4^{abc}	0.40 ± 0.01^{abc}	0.8 ± 0.3^{a}	4.39 ± 0.09^{ab}
CDC Walrus	CWES	13.5 ± 0.5^{bcde}	0.37 ± 0.03^{abc}	0.8 ± 0.2^{a}	4.42 ± 0.43^{ab}
AAC Iceberg	CWHWS	13.2 ± 0.9^{bcde}	0.35 ± 0.02^{abc}	0.6 ± 0.4^{a}	4.42 ± 0.44^{ab}
CDC Whitewood	CWHWS	13.2 ± 0.6^{bcde}	0.34 ± 0.01^{a}	0.6 ± 0.2^{a}	4.11 ± 0.53^{ab}
Whitehawk	CWHWS	13.0 ± 0.2^{bcde}	0.38 ± 0.01^{abc}	0.8 ± 0.1^{a}	4.84 ± 0.37^{ab}
AAC Brandon	CWRS	13.1 ± 0.7^{bcde}	0.37 ± 0.03^{abc}	0.7 ± 0.1^{a}	4.86 ± 0.79^{ab}
AAC Redwater	CWRS	13.8 ± 1.1^{cde}	0.39 ± 0.02^{abc}	0.7 ± 0.2^{a}	4.47 ± 0.91^{ab}
Carberry	**CWRS**	**13.5 ± 0.7^{bcde}**	**0.37 ± 0.01^{abc}**	**0.6 ± 0.2^{a}**	**4.74 ± 0.28^{ab}**
CDC Plentiful	CWRS	13.8 ± 0.3^{cde}	0.37 ± 0.01^{abc}	0.7 ± 0.3^{a}	3.91 ± 0.63^{ab}
CDC Stanley	CWRS	13.4 ± 0.6^{bcde}	0.36 ± 0.02^{abc}	0.6 ± 0.3^{a}	3.64 ± 0.61^{a}
CDC Titanium	CWRS	14.1 ± 0.6^{cde}	0.41 ± 0.03^{abc}	0.8 ± 0.4^{a}	4.27 ± 0.87^{ab}
CDC Utmost	CWRS	13.6 ± 0.6^{bcde}	0.35 ± 0.01^{ab}	0.7 ± 0.1^{a}	3.88 ± 0.52^{ab}
Glenn	**CWRS**	**12.7 ± 0.5^{bcd}**	**0.36 ± 0.03^{abc}**	**0.6 ± 0.3^{a}**	**5.62 ± 0.19^{b}**
Parata	CWRS	13.6 ± 0.7^{bcde}	0.36 ± 0.01^{abc}	0.7 ± 0.5^{a}	4.46 ± 0.94^{ab}
Roblin	CWRS	14.8 ± 0.6^{e}	0.35 ± 0.01^{abc}	0.7 ± 0.3^{a}	3.89 ± 0.10^{ab}
Shaw VB	CWRS	13.4 ± 0.4^{bcde}	0.41 ± 0.03^{bc}	0.6 ± 0.4^{a}	4.79 ± 0.73^{ab}
Stettler	CWRS	13.6 ± 0.2^{bcde}	0.42 ± 0.03^{bc}	0.6 ± 0.2^{a}	4.29 ± 0.81^{ab}
CDC Kinley	CWSP	13.6 ± 0.5^{bcde}	0.38 ± 0.03^{abc}	0.6 ± 0.3^{a}	4.60 ± 0.17^{ab}
Pasteur	CWSP	10.5 ± 0.3^{a}	0.42 ± 0.02^{c}	0.6 ± 0.3^{a}	5.60 ± 0.14^{b}
Average	**-**	**13.2**	**0.38**	**0.7**	**4.59**
Min/Max	**-**	**10.5/14.8**	**0.34/0.42**	**0.6/0.8**	**3.64/5.62**

Data within the same colunm with different letters are significantly different (p<0.05).

The mean falling number (FN) was 364 s (varying from 285 s - AAC Iceberg to 390 s - Shaw VB), with white wheat cultivars susceptible to pre-harvest sprouting, such as AAC Iceberg have the least FN at 285 s and Shaw VB with the highest at 390 s (Table 3.4). As FN is inversely proportional to enzymatic activity, values suggest there may be more enzymatic activity occurring within the crops used in the present study relative to those grown in other places on the Western (410 s) and Eastern (435 s) Prairies in the same crop year (Causgrove, et al., 2004; CGC, 2016). The mean HI for the 25 cultivars was 58 (ranging from 47 (Roblin) to 74 (Glenn), both belonging to the CWRS (Table 3.4). A high grain hardness index indicates that more strength is needed to crush the kernel, causing increased starch damage in the flour and higher water absorption. A significant correlation was found betw grain hardness and damage starch (r = -0.57, p<0.01) and micro-doughLAB absorption (r = 0.41, p<0.01).

Table 3.4. Wheat quality properties for twenty-five cultivars. Data represents the mean ± standard deviation from 3 biological replications (n = 3).

Cultivar	Wheat Class	FN (s)	Hardness Index (SKCS) (HI)	WG (%)	DG (%)	GI (%)
Harvest	CNHR	380 ± 37^{b}	64 ± 3^{gh}	41.1 ± 3.5^{efg}	14.3 ± 1.0^{bcd}	70.3 ± 2.4^{ab}
Lillian	CNHR	380 ± 12^{b}	62± 1^{fgh}	43.5 ± 1.9^{g}	15.1 ± 0.7^{d}	59.8 ± 2.9^{a}
McKenzie	CNHR	348 ± 26^{ab}	65 ± 3^{h}	38.2 ± 0.8^{bcdefg}	13.3 ± 0.4^{bcd}	83.9 ± 5.9^{cde}
Pembina	CNHR	352 ± 8^{ab}	55 ± 1^{bcdef}	35.4 ± 1.6^{bcd}	13.6 ± 1.5^{bcd}	96.0 ± 1.0^{fg}
Unity VB	CNHR	384 ± 20^{b}	61 ± 2^{fgh}	36.7 ± 1.4^{bcde}	12.6 ± 0.4^{bc}	83.8 ± 8.3^{cd}
5702PR	CPSR	376 ± 9^{b}	50 ± 0^{ab}	32.9 ± 2.6^{ab}	12.2 ± 1.1^{b}	98.2 ± 1.2^{g}
CDC Terrain	CPSR	386 ± 17^{b}	49 ± 2^{ab}	34.6 ± 1.4^{abc}	12.5 ± 0.7^{b}	95.7 ± 0.9^{fg}
CDC Walrus	CWES	364 ± 16^{b}	52 ± 1^{abc}	36.6 ± 1.5^{bcde}	13.6 ± 0.5^{bcd}	99.0 ± 0.5^{g}
AAC Iceberg	CWHWS	285 ± 36^{a}	53 ± 1^{abcd}	37.8 ± 2.8^{bcdef}	13.4 ± 0.9^{bcd}	96.1 ± 2.0^{fg}
CDC Whitewood	CWHWS	369 ± 28^{b}	54 ± 3^{abcde}	36.3 ± 1.2^{bcde}	12.9 ± 0.5^{bcd}	95.6 ± 2.2^{efg}
Whitehawk	CWHWS	390 ± 20^{b}	64 ± 4^{gh}	36.4 ± 1.2^{bcde}	13.4 ± 0.9^{bcd}	97.9 ± 0.8^{g}
AAC Brandon	CWRS	343 ± 24^{ab}	60 ± 2^{defgh}	38.0 ± 0.8^{bcdefg}	13.6 ± 0.6^{bcd}	88.3 ± 1.9^{cdefg}
AAC Redwater	CWRS	382 ± 21^{b}	60 ± 4^{efgh}	41.5 ± 3.1^{efg}	14.3 ± 1.0^{bcd}	82.8 ± 5.1^{cd}
Carberry	**CWRS**	**334 ± 26^{ab}**	**59 ± 1^{defgh}**	**37.7 ± 1.2^{bcdef}**	**13.5 ± 0.4^{bcd}**	**93.3 ± 2.5^{defg}**
CDC Plentiful	CWRS	372 ± 21^{b}	60 ± 1^{defgh}	39.2 ± 0.5^{cdefg}	13.6 ± 0.2^{bcd}	91.8 ± 2.5^{defg}
CDC Stanley	CWRS	376 ± 7^{b}	47 ± 2^{a}	39.0 ± 1.9^{cdefg}	13.6 ± 0.7^{bcd}	90.1 ± 0.3^{defg}
CDC Titanium	CWRS	372 ± 16^{b}	58 ± 0^{cdefg}	40.6 ± 1.0^{defg}	14.3 ± 0.4^{bcd}	96.1 ± 1.2^{fg}
CDC Utmost	CWRS	379 ± 27^{b}	55 ± 4^{bcdef}	40.0 ± 1.4^{cdefg}	14.2 ± 0.6^{bcd}	83.3 ± 9.1^{cd}
Glenn	**CWRS**	**339 ± 21^{ab}**	**74 ± 2^{i}**	**34.9 ± 1.4^{abc}**	**12.7 ± 0.7^{bc}**	**99.0 ± 0.9^{g}**
Parata	CWRS	383 ± 18^{b}	60 ± 3^{efgh}	39.0 ± 2.1^{cdefg}	13.5 ± 0.5^{bcd}	91.2 ± 2.1^{defg}
Roblin	CWRS	351 ± 28^{ab}	47 ± 1^{a}	43.3 ± 2.5^{fg}	14.9 ± 1.4^{cd}	93.6 ± 1.9^{defg}
Shaw VB	CWRS	390 ± 23	63 ± 2^{gh}	39.1 ± 0.9^{cdefg}	13.8 ± 0.3^{bcd}	83.8 ± 5.9^{cd}
Stettler	CWRS	346 ± 23^{ab}	62 ± 2^{fgh}	40.6 ± 1.5^{defg}	14.2 ± 0.4^{bcd}	77.6 ± 4.0^{bc}
CDC Kinley	CWSP	363 ± 9^{b}	56 ± 3^{bcdef}	37.9 ± 0.4^{bcdefg}	13.4 ± 0.1^{bcd}	91.1 ± 2.0^{defg}
Pasteur	CWSP	359 ± 24^{b}	66 ± 1^{h}	29.5 ± 0.8^{a}	9.9 ± 0.3^{a}	85.5 ± 5.1^{cdef}
Average	**-**	**364**	**58**	**38.0**	**13.5**	**88.9**
Min/Max	**-**	**285/390**	**47/74**	**29.5/43.5**	**9.9/15.1**	**59.8/99.0**

Abbreviations: Falling number (FN); single kernel characterization system (SKCS); hardness index (HI); wet gluten (WG); dry gluten (DG); gluten index (GI).
Data within the same colunm with different letters are significantly different (p<0.05).

Dry gluten (DG) content had a mean of 13.5% (ranging from 9.9% - Pasteur to 15.1% - Lillian). The wet gluten (WG) was found to have a mean of 38.0% (ranging from 29.5% - Pasteur to 43.5% - Lillian). The gluten index (GI) according to the CGC reported across the Prairies in 2016, GI was on average 89-92. For the cultivars tested, the GI was found to have a mean of 88.9, ranging from 59.8 (Lillian) to 99 (Glenn). The cultivars with lowest gluten indices were Harvest (70.3), Lillian (59.8) and Stettler (77.6), therefore they may produce dough of poor quality. Whereas all other cultivars were above 80, thus better dough handling properties could be expected. In addition, positive correlation was found between GI and protein content (r = 0.23, p<0.05), and rheology parameters, such as J_{max} (r = 0.67, p<0.01) and

tan δ (r = 0.58, p<0.01). Negative correlation was found to both the dry (r = -0.30, p<0.01) and wet (r = -0.48, p<0.01) gluten contents, and |G*| (r = -0.73, p<0.01). The PCA of compositional and quality data (Figure A3.2 Appendix) showed no clear discernible clusters, with the exception of Lillian and Pasteur which were distinct from the rest. The protein content being towards the highest (14.4%, Lillian) and lowest (10.5%, Pasteur), in addition to high content of ash (0.42%), may have contributed to set them apart from the rest of the cultivars.

The flour quality depends on several characteristics that directly influence the end products, i.e., baking performance. The existing to determine those parameters, require specific sample size, equipment, and resources. Therefore, there is a high demand from breeding programs for rapid and reliable tests, especially, for early generation screen, which requires test methods that need only a small amount of sample, and quick performing time (Xiao, et al., 2006). The SRC test uses four solvents as a means of predicting their contribution to the flour's overall quality. Flour SRC results to different solvents are presented on Table 3.5 and varied significantly within the cultivars (p<0.05).

The distilled water is associated with the contributions from all flour components, reflecting the ability of flour to hold water. The average of retention capacity of 67.2% (ranging from 62.5% – 5702PR to 71.7% – Glenn) were given, which represent highest ABS for Glenn as the correlation between ABS and SRC water solvent was significant (p<0.01), r = 0.56 (Table A3.1, Appendix). In the sucrose solution (which is associated with the contributions from water-soluble pentosans), the retention capacity value was found to be 102.7% (ranging from 93.6% – 5702PR to 108.5% – Pasteur), this result is in line with previous work reported by Xiao et al. (2006) on hard wheat flour (88.1–142.1%). Sodium bicarbonate solution is sensitive to swelling of damaged starch and pentosans in flour, it had a mean value of 79.2% (ranging from 72.3% – 5702PR to 90.1% – Pasteur). As this solvent is related to damaged starch (DS), higher values can indicate higher amounts of damaged starch, correlations were significant (p<0.01) between DS and SRC sodium carbonate. For instance, Pasteur had one of the highest damaged starch values (5.60%) and 5702PR was closest to the lower damaged starch (4.33%) (Table 3.5). Lactic acid is associated with the contributions from gluten proteins, indicating dough strength from the glutenin subunits swelling (Kweon et al., 2011). The mean value was 149.8% (ranging from 128.5% – Pasteur to 171.3% – Roblin), thus higher values of SRC for lactic acid reflect stronger dough properties. In addition, usually, spring wheat flour contains a higher protein content level than winter wheat flour (Maghirang et al., 2006; Hammed, et al., 2015).

Table 3.5. Solvent retention capacity (SRC) and gluten performance index (GPI) mean and standard deviation for twenty-five wheat cultivars (n=3; $p<0.05$).

Cultivar	Wheat Class	dd H_2O (%)	Sucrose (%)	Sodium carbonate (%)	Lactic Acid (%)	GPI
Harvest	CNHR	68.31 ± 0.87^{defg}	96.57 ± 4.55^{ab}	78.47 ± 0.52^{bcdef}	135.96 ± 2.02^{abc}	0.78 ± 0.02^{bcde}
Lillian	CNHR	70.89 ± 0.89^{hi}	108.32 ± 5.92^{b}	85.76 ± 2.34^{i}	142.24 ± 0.57^{bcd}	0.73 ± 0.02^{b}
Mckenzie	CNHR	68.47 ± 0.52^{defgh}	98.68 ± 4.17^{ab}	80.59 ± 0.41^{efg}	142.78 ± 2.07^{bcd}	0.80 ± 0.03^{bcdef}
Pembina	CNHR	65.02 ± 0.69^{bc}	104.22 ± 1.36^{ab}	78.46 ± 1.12^{bcdef}	158.75 ± 2.53^{gh}	0.87 ± 0.01^{fghi}
Unity VB	CNHR	69.02 ± 0.33^{efgh}	104.84 ± 1.17^{ab}	81.59 ± 0.56^{fgh}	142.95 ± 1.48^{cd}	0.77 ± 0.01^{bcd}
5702PR	CPSR	62.55 ± 0.76^{a}	93.59 ± 5.78^{a}	72.33 ± 0.66^{a}	150.21 ± 1.98^{def}	0.90 ± 0.02^{hi}
CDC Terrain	CPSR	65.13 ± 1.36^{bc}	100.96 ± 1.66^{ab}	75.25 ± 1.77^{ab}	128.63 ± 3.16^{a}	0.73 ± 0.02^{b}
CDC Walrus	CWES	66.35 ± 0.93^{bcd}	99.65 ± 5.14^{ab}	77.08 ± 0.99^{bcde}	165.50 ± 5.26^{hij}	0.94 ± 0.05^{i}
AAC Iceberg	CWHWS	66.85 ± 1.53^{cde}	104.87 ± 4.14^{ab}	78.11 ± 0.84^{bcdef}	162.08 ± 3.46^{hi}	0.89 ± 0.04^{ghi}
CDC Whitewood	CWHWS	64.96 ± 0.46^{abc}	106.40 ± 1.40^{ab}	75.90 ± 0.76^{abc}	$159.27 \pm 3.52g^{hi}$	0.88 ± 0.02^{ghi}
Whitehawk	CWHWS	66.28 ± 0.90^{bcd}	97.25 ± 4.58^{ab}	77.52 ± 1.73^{bcde}	146.21 ± 1.24^{de}	0.84 ± 0.02^{defgh}
AAC Brandon	CWRS	69.53 ± 0.49^{fghi}	103.95 ± 5.35^{ab}	82.64 ± 1.41^{ghi}	145.52 ± 1.77^{de}	0.78 ± 0.02^{bcde}
AAC Redwater	CWRS	67.38 ± 0.50^{cdef}	102.19 ± 5.41^{ab}	79.85 ± 0.44^{defg}	148.42 ± 1.79^{de}	0.82 ± 0.03^{cdefg}
Carberry	**CWRS**	$\mathbf{66.82 \pm 0.88^{cde}}$	$\mathbf{103.96 \pm 1.10^{ab}}$	$\mathbf{76.87 \pm 0.32^{bcd}}$	$\mathbf{159.22 \pm 2.76^{ghi}}$	$\mathbf{0.88 \pm 0.01^{ghi}}$
CDC Plentiful	CWRS	67.37 ± 0.46^{cdef}	104.06 ± 5.96^{ab}	79.24 ± 0.36^{cdefg}	161.66 ± 0.22^{hi}	0.88 ± 0.03^{ghi}
CDC Stanley	CWRS	63.92 ± 0.73^{ab}	100.18 ± 6.44^{ab}	75.24 ± 1.46^{ab}	152.26 ± 3.70^{efg}	0.87 ± 0.04^{fghi}
CDC Titanium	CWRS	68.30 ± 0.85^{defg}	106.27 ± 4.75^{ab}	80.68 ± 1.07^{efg}	158.09 ± 2.33^{fgh}	0.85 ± 0.03^{efgh}
CDC Utmost	CWRS	66.78 ± 0.51^{cde}	102.21 ± 5.50^{ab}	79.25 ± 1.40^{cdefg}	148.23 ± 0.90^{de}	0.82 ± 0.02^{cdefg}
Glenn	**CWRS**	$\mathbf{71.66 \pm 0.80^{i}}$	$\mathbf{105.64 \pm 4.21^{ab}}$	$\mathbf{84.46 \pm 2.20^{hi}}$	$\mathbf{167.38 \pm 1.87^{ij}}$	$\mathbf{0.88 \pm 0.02^{ghi}}$
Parata	CWRS	67.28 ± 0.31^{cdef}	106.12 ± 2.13^{ab}	78.82 ± 0.16^{bcdef}	152.47 ± 1.41^{efg}	0.83 ± 0.01^{defg}
Roblin	CWRS	66.21 ± 0.69^{bcd}	107.48 ± 5.85^{ab}	77.64 ± 2.05^{bcde}	171.29 ± 2.57^{j}	0.92 ± 0.01^{i}
Shaw VB	CWRS	67.23 ± 0.77^{cdef}	100.93 ± 0.54^{ab}	78.76 ± 0.47^{bcdef}	134.67 ± 4.06^{ab}	0.75 ± 0.02^{bc}
Stettler	CWRS	66.04 ± 0.36^{bcd}	96.95 ± 5.21^{ab}	77.02 ± 0.54^{bcde}	135.07 ± 0.99^{abc}	0.78 ± 0.02^{bcde}
CDC Kinley	CWSP	66.34 ± 0.24^{bcd}	103.93 ± 2.29^{ab}	77.77 ± 0.29^{bcde}	148.20 ± 3.80^{de}	0.82 ± 0.02^{cdefg}
Pasteur	CWSP	70.52 ± 1.02^{ghi}	108.53 ± 5.90^{b}	90.07 ± 0.49^{j}	128.53 ± 2.76^{a}	0.65 ± 0.01^{a}
Average	**-**	**67.17**	**102.71**	**79.18**	**149.82**	**0.82**
Min/Max	**-**	**62.55/71.66**	**93.59/108.53**	**72.33/90.07**	**128.53/171.29**	**0.65/0.94**

Data within the same colunm with different letters are significantly different ($p<0.05$).

Significant correlations were found between SRC lactic acid and protein content, STA, and ETP ($p<0.01$), and MDT, PKH, DG and GI ($p<0.05$). All of those parameters are related to dough strength. Same trend was found for the gluten performance index (GPI) values varied significantly among flours obtained from different cultivars with mean value of 0.82 (ranging from 0.65 – Pasteur to 0.94 – CDC Walrus). As GPI estimates the overall performance of the gluten proteins within the dough network, a higher value is expected for stronger cultivars, such as CDC Walrus (Kweon et al., 2011). The targeted ranges for wheat flours for solvent retentions

are 65-70% for water, 105-115% for sucrose, >140% for lactic acid, 80-90% for sodium carbonate, and 0.75 for the gluten performance index (www.uswheat.org). Both cultivars checks, Carberry and Glenn, had the same GPI (0.88), which indicates a high overall gluten performance for CWRS cultivar. Principal component analysis based on the SRC data (Figure A3.3, Appendix suggested that Lillian, Pasteur, Shaw VB, Unity VB, AAC Brandon, Harvest and McKenzie were closely related, all having gluten performance indices ≤80. It also demonstrated other similar clusters, for instance, Glenn, Stettler, AAC Redwater, and CDC Utmost. And CDC Plentiful, CDC Walrus, CDC Stanley, AAC Iceberg, 5702PR and Roblin. In addition, Carberry, Pembina, and CDC Whitewood were similar, whereas CDC Terrain, Parata and CDC Kinley were distinctly different from one another.

3.4.3. Micro-doughLAB analysis

Micro-doughLAB tests results are presented in Table 3.6. The ABS, was found to have a mean of 60.1%, ranging from 55.6% (5702PR) to 62.6% (Lillian). As ABS which is the water needed for the dough to reach a defined consistency, it was found to be positively correlated with protein content ($r = 0.61$, $p<0.01$), grain hardness ($r = 0.41$, $p<0.01$), wet gluten ($r = 0.69$, $p<0.01$) and dry gluten ($r = 0.56$, $p<0.01$), and negatively correlated with the gluten performance index ($r = -0.47$, $p<0.01$). Other studies have found that protein content and gluten properties affect ABS in wheat flour (Hammed et al., 2015). The dough stability indicates that the dough maintained at least mixing torque index of 500 BU, it was found to average 7.3 min (ranging from 3.6 min – Lillian to 15.9 min – CDC Walrus), which fell within the same range reported by Hammed et al. (2015) analyzing hard red spring wheat (7.3–16.2 min). Higher STA values were expected for CDC Walrus, as this cultivar is part of the CWES wheat class. The wheat checks for CWRS class had values in between the highest and lowest stability, being Carberry 6.1 min and Glenn 10.1 min. In general, wheat cultivar flours which have lower ABS and shorter STA times tend to have weaker gluten properties. In contrast, stronger flours tend to have higher absorption and longer stability times (Maghirang et al., 2006). The DDT was found to have a mean of 4.7 min (ranging from 2.9 min - Lillian to 8.3 min - CDC Walrus). It is expected that stronger cultivars have higher time to reach maximum dough consistency during mixing, due to strong gluten network formation until it starts breaking down from over-mixing . This tolerance of mixing is determined by mixing tolerance index (MTI) which was found to have a mean of 52 (ranging from 25 - Whitehawk to 71 - CDC Utmost). Similar to the gluten index, Harvest, Lillian and Stettler tended to form weaker doughs (shorter dough

stability times), along with Pasteur, McKenzie and Parata. Doughs prepared from these flours also became softer faster as over-mixing occurred (MTI).

Table 3.6. Micro-doughLAB parameters for twenty-five wheat cultivars. Data represents the mean ± standard deviation from 3 biological replications (n = 3).

Cultivar	Wheat Class	ABS (%)	STA (min)	DDT (min)	MTI (torq, FU)
Harvest	CNHR	61.9 ± 0.6^{ij}	4.6 ± 1.2^{ab}	3.3 ± 0.2^{abc}	65 ± 17^{def}
Lillian	CNHR	62.6 ± 0.2^{j}	3.6 ± 0.8^{a}	2.9 ± 0.5^{a}	68 ± 13^{ef}
McKenzie	CNHR	59.8 ± 0.2^{defgh}	5.0 ± 0.7^{abcd}	3.1 ± 0.4^{ab}	62 ± 8^{cdef}
Pembina	CNHR	57.1 ± 0.6^{ab}	9.2 ± 2.7^{cdef}	5.3 ± 1.2^{bcdef}	40 ± 13^{abcde}
Unity VB	CNHR	59.7 ± 0.4^{cdefg}	4.1 ± 1.1^{ab}	3.1 ± 0.3^{ab}	70 ± 15^{f}
5702PR	CPSR	55.6 ± 0.9^{a}	12.9 ± 2.1^{fg}	5.9 ± 1.1^{def}	30 ± 9^{ab}
CDC Kinley	CPSR	60.1 ± 1.0^{defghi}	7.1 ± 0.9^{abcde}	4.8 ± 0.6^{abcde}	47 ± 8^{abcdef}
CDC Walrus	CWES	59.1 ± 0.2^{cde}	15.9 ± 0.9^{g}	8.3 ± 2.3^{g}	33 ± 19^{abc}
AAC Iceberg	CWHWS	60.6 ± 0.5^{efghi}	5.9 ± 1.2^{abcde}	4.5 ± 0.6^{abcde}	65 ± 13^{def}
CDC Whitewood	CWHWS	59.6 ± 0.8^{cdef}	7.8 ± 0.3^{abcde}	5.5 ± 0.7^{cdef}	47 ± 3^{abcdef}
Whitehawk	CWHWS	60.6 ± 0.2^{efghi}	13.2 ± 3.5^{fg}	6.4 ± 0.6^{efg}	25 ± 5^{a}
AAC Brandon	CWRS	60.9 ± 0.9^{fghij}	5.9 ± 0.0^{abcde}	4.3 ± 0.4^{abcde}	57 ± 3^{bcdef}
AAC Redwater	CWRS	61.5 ± 0.9^{hij}	5.4 ± 0.9^{abcd}	4.1 ± 0.4^{abcd}	57 ± 10^{bcdef}
Carberry	**CWRS**	$\mathbf{59.8 \pm 0.1^{defgh}}$	$\mathbf{6.1 \pm 0.7^{abcde}}$	$\mathbf{4.2 \pm 0.3^{abcde}}$	$\mathbf{62 \pm 6^{cdef}}$
CDC Plentiful	CWRS	59.9 ± 0.6^{defgh}	7.6 ± 0.4^{abcde}	4.9 ± 0.4^{abcde}	45 ± 0^{abcdef}
CDC Stanley	CWRS	58.8 ± 0.1^{bcd}	5.7 ± 0.6^{abcde}	3.8 ± 0.4^{abcd}	57 ± 6^{bcdef}
CDC Titanium	CWRS	61.1 ± 0.4^{fghij}	9.4 ± 1.1^{def}	5.7 ± 0.5^{def}	37 ± 8^{abcd}
CDC Utmost	CWRS	60.2 ± 0.9^{defghi}	4.8 ± 0.3^{abc}	3.9 ± 0.3^{abcd}	72 ± 6^{f}
Glenn	**CWRS**	$\mathbf{61.2 \pm 0.3^{fghij}}$	$\mathbf{10.1 \pm 1.5^{ef}}$	$\mathbf{7.5 \pm 1.1^{fg}}$	$\mathbf{45 \pm 5^{abcdef}}$
Parata	CWRS	61.4 ± 0.1^{ghij}	7.0 ± 0.6^{abcde}	4.9 ± 0.2^{abcde}	50 ± 5^{abcdef}
Roblin	CWRS	61.5 ± 0.9^{ghij}	10.0 ± 3.1^{ef}	5.7 ± 0.5^{def}	33 ± 10^{abc}
Shaw VB	CWRS	61.0 ± 0.6^{fghij}	4.7 ± 0.6^{ab}	3.7 ± 0.3^{abcd}	67 ± 6^{ef}
Stettler	CWRS	60.8 ± 0.4^{efghi}	4.7 ± 0.7^{ab}	3.2 ± 0.3^{ab}	65 ± 5^{def}
CDC Terrain	CWSP	58.0 ± 0.4^{bc}	8.3 ± 1.4^{bcde}	5.3 ± 0.5^{bcdef}	45 ± 10^{abcdef}
Pasteur	CWSP	58.7 ± 0.4^{bcd}	4.2 ± 0.4^{ab}	3.1 ± 0.6^{ab}	70 ± 5^{f}
Average	**-**	**60.1**	**7.3**	**4.7**	**52.5**
Min/Max	**-**	**55.6/62.6**	**3.6/15.9**	**2.9/8.3**	**25.0/71.7**

Abbreviations: absorption (ABS); stability (STA); dough development time (DDT); mixing tolerance index (MTI).
Data within the same colunm with different letters are significantly different ($p<0.05$).

PCA revealed that AAC Redwater, AAC Brandon, Lillian, Harvest, Shaw VB, Stettler, and AAC Iceberg formed a cluster, which indicates similar flour properties. Furthermore, cultivars McKenzie, CDC Utmost, Unity VB, Pasteur were similar to Carberry the lower strength check. In contrast, Roblin, CDC Titanium, CDC Kinley, Whitehawk, CDC Plentiful, CDC Whitewood, CDC Walrus, and Glenn formed another cluster, which may characterize them as stronger dough cultivars. Cultivars 5702PR, Pembina and CDC Terrain were in the same cluster, whereas Parata and CDC Stanley were distinctly different from one another (Figure A3.4, Appendix).

3.4.4. Mixograph analysis

Mixograph parameters tested are presented on Table 3.7, where mixing time to peak (MTP) was found to have a mean of 3.3 min, ranging from 2.4 min (Harvest) to 4.6 min (CDC Walrus). The difference in mixing time between the cultivars Carberry and Glenn was 0.8 min (3.3 and 4.1 min, respectively). Peak dough resistance (PKH) was found to have a mean of 60.0% (ranging from 54.8% - CDC Kinley to 66.0% - Roblin), which indicates that Roblin may be the most tolerant to over-mixing. Carberry and Glenn had a small variation in PKH ~ 1.7%, indicating that CWRS cultivars have higher tolerance to over-mixing. Band width at peak resistance (%) was found to have a mean of 32.9% (ranging from 26.6 -Pasteur to 39.9% - 5702PR). It is expected that stronger cultivars require higher energy to develop the dough and, as consequence, longer mixing time (Maghirang et al., 2006).

Table 3.7. Mixograph parameters for twenty-five wheat cultivars. Data represents the mean ± standard deviation from 3 biological replications (n = 3).

Cultivar	Wheat Class	MDT (Min)	PKH (%)	BWP (%)	WIP (% tq.min)
Harvest	CNHR	2.4 ± 0.4^{a}	64.3 ± 5.2^{ab}	38.2 ± 3.1^{a}	103 ± 7^{a}
Lillian	CNHR	2.8 ± 0.1^{ab}	58.7 ± 2.6^{ab}	27.3 ± 2.0^{a}	117 ± 11^{abc}
McKenzie	CNHR	3.2 ± 0.3^{abcde}	57.8 ± 1.4^{ab}	31.1 ± 1.2^{a}	122 ± 12^{abc}
Pembina	CNHR	3.8 ± 0.3^{defg}	56.2 ± 1.7^{ab}	33.4 ± 3.0^{a}	160 ± 15^{cdefg}
Unity VB	CNHR	2.9 ± 0.1^{abc}	56.6 ± 3.1^{ab}	33.8 ± 3.5^{a}	112 ± 2^{ab}
5702PR	CPSR	3.9 ± 0.2^{efg}	55.0 ± 4.2^{a}	39.9 ± 7.5^{a}	159 ± 12^{cdefg}
CDC Terrain	CPSR	4.1 ± 0.5^{fg}	61.2 ± 4.1^{ab}	26.6 ± 5.8^{a}	182 ± 21^{fgh}
CDC Walrus	CWES	4.6 ± 0.1^{g}	62.8 ± 0.6^{ab}	32.5 ± 0.4^{a}	208 ± 14^{h}
AAC Iceberg	CWHWS	3.2 ± 0.2^{abcdef}	61.7 ± 4.5^{ab}	28.9 ± 2.7^{a}	152 ± 23^{bcdefg}
CDC Whitewood	CWHWS	3.4 ± 0.2^{bcdef}	60.1 ± 4.9^{ab}	36.3 ± 6.5^{a}	151 ± 23^{bcdefg}
Whitehawk	CWHWS	4.1 ± 0.2^{fg}	59.5 ± 4.1^{ab}	39.0 ± 4.9^{a}	177 ± 4^{efgh}
AAC Brandon	CWRS	3.4 ± 0.2^{bcdef}	57.3 ± 1.5^{ab}	32.5 ± 7.7^{a}	140 ± 19^{abcdef}
AAC Redwater	CWRS	2.8 ± 0.4^{ab}	62.1 ± 2.9^{ab}	32.4 ± 3.8^{a}	123 ± 20^{abc}
Carberry	**CWRS**	$\mathbf{3.3 \pm 0.1^{bcdef}}$	$\mathbf{60.3 \pm 1.6^{ab}}$	$\mathbf{35.5 \pm 4.0^{a}}$	$\mathbf{133 \pm 11^{abcd}}$
CDC Plentiful	CWRS	3.0 ± 0.1^{abc}	61.8 ± 2.7^{ab}	33.7 ± 0.8^{a}	132 ± 6^{abcd}
CDC Stanley	CWRS	2.9 ± 0.3^{abc}	57.8 ± 2.6^{ab}	34.0 ± 0.2^{a}	125 ± 8^{abc}
CDC Titanium	CWRS	4.1 ± 0.3^{fg}	63.6 ± 4.1^{ab}	30.3 ± 2.5^{a}	192 ± 25^{gh}
CDC Utmost	CWRS	2.9 ± 0.4^{abc}	63.5 ± 3.2	31.6 ± 5.2^{a}	128 ± 11^{abc}
Glenn	**CWRS**	$\mathbf{4.1 \pm 0.1^{fg}}$	$\mathbf{62.0 \pm 1.2^{ab}}$	$\mathbf{36.7 \pm 5.0^{a}}$	$\mathbf{175 \pm 7^{defgh}}$
Parata	CWRS	3.2 ± 0.4^{abcde}	59.6 ± 1.0^{ab}	31.9 ± 5.3^{a}	137 ± 13^{abcde}
Roblin	CWRS	3.2 ± 0.3^{abcde}	66.0 ± 1.9^{b}	30.4 ± 0.5^{a}	151 ± 5^{bcdefg}
Shaw VB	CWRS	3.1 ± 0.3^{abcd}	60.9 ± 0.2^{ab}	32.7 ± 6.2^{a}	130 ± 10^{abc}
Stettler	CWRS	2.8 ± 0.1^{ab}	60.1 ± 2.3^{ab}	33.2 ± 2.1^{a}	114 ± 5^{ab}
CDC Kinley	CWSP	3.5 ± 0.0^{bcdef}	54.8 ± 6.1^{a}	34.3 ± 2.2^{a}	140 ± 10^{abcdef}
Pasteur	CWSP	3.7 ± 0.3^{cdef}	56.2 ± 0.4^{ab}	26.6 ± 6.1^{a}	143 ± 13^{abcdef}
Average	**-**	**3.3**	**60.0**	**32.9**	**144**
Min/Max	**-**	**2.4/4.7**	**54.8/66.0**	**26.6/39.9**	**103/208**

Abbreviations: mixograph development time (MDT); peak height (PKH); bandwidth to peak (BWP); work input to peak (WIP). Data within the same colunm with different letters are significantly different ($p<0.05$).

Therefore, the work input to peak (WIP) for dough development was found to have a mean of 144% tq.min (ranging from 103% tq.min - Harvest to 208% tq.min - CDC Walrus), which is in accordance to the MTP, with Harvest being the lowest and CDC Walrus the highest. Based on PCA results (Figure A3.5, Appendix), Glenn formed a cluster with CDC Walrus, CDC Terrain, and CDC Titanium, as the strongest end for mixograph analysis. In contrast, Carberry, formed a cluster with CDC Stanley, Unity VB, Stettler, CDC Whitewood, and McKenzie, representing the weaker cultivars. The rest of the genotypes formed clusters in different quadrants, such as Pasteur, AAC Brandon, CDC Kinley, Whitehawk, and Pembina, in one and Roblin, CDC Utmost, AAC Iceberg, Shaw VB, AAC Redwater, Lillian, Parata, CDC Plentiful, and Harvest in another.

3.4.5. Oscillatory shear rheometry and creep compliance

Rheological properties of dough can be challenging to characterize, due to its complexity, non-linear behavior, and time dependent viscoelastic system. Because of the controlled stress and strain conditions in oscillatory measurements with small amplitudes do not highly affect or destroy the dough structure, its application showed to be appropriate (Jekle and Becker, 2011). Oscillatory shear rheometry and creep compliances data for dough prepared from the 25 cultivars is presented in Table 3.8. Dough samples for complex modulus (|G*|) were found to have a mean of 12.6 kPa (from 9.2 kPa, Shaw VB to 18.8 kPa, 5702PR), whereas loss tangent (tan δ) values were found to have a mean of 0.37, ranging from 0.33 (5702PR, Glenn, and Whitehawk) to 0.42 (Lillian). Song and Zheng (2007) reported that cultivars with lower tan δ tended to represent stronger wheat cultivars, whereas the opposite was observed for weaker wheat cultivars. These findings are in line with values accounted for Carberry and Glenn (0.38 and 0.33, respectively), the lowest and highest strength range for CWRS wheat check. The |G*| was found to be positively correlated to the dry gluten content ($r = 0.73$, $p<0.01$) and damaged starch ($r = 0.27$, $p<0.05$), and negatively correlated with the protein ($r = -0.77$, $p<0.01$) and wet gluten ($r = -0.83$, $p<0.01$) contents. On the other hand, tan δ was found to be positively correlated with protein content ($r = 0.61$, $p<0.01$), dry gluten content ($r = 0.58$, $p<0.01$) and wet gluten content ($r = 0.72$, $p<0.01$) and negatively correlated to damage starch content ($r = - 0.26$, $p<0.05$) and the gluten index ($r = -0.63$, $p<0.01$).

During the creep recovery test, an instantaneous stress is applied to the dough and the change in strain is measured over time. Typically, the creep phase is followed by a recovery phase (i.e., applied stress is removed) (Bockstaele et al., 2008). The maximum creep compliance (J_{max}) and the relative elasticity (J_{el}) during a creep recovery measurement are

presented in Table 3.8. Creep recovery tests have shown good correlations to the baking properties, presenting a high correlation between maximum recovery strain and bread volume (Wang and Sun, 2002). Usually long relaxation times are well correlated with better breadmaking quality parameters (Figueroa, et al., 2013). J_{max} was found to have a mean value of 1.26 mPa^{-1} (ranging from 0.57 mPa^{-1} - 5702PR to 2.60 mPa^{-1} - Stettler), and a mean J_{el} value of 0.65 (ranging from 0.50 - Roblin to 0.71 - AAC Iceberg, CDC Plentiful, and Pembina). Significant correlations (p<0.01 and p<0.05) were found between J_{max} and all mixograph parameters, except for BTW. In addition, J_{max} was found to be positively correlated with protein (r = 0.65; p<0.01), wet gluten (r = 0.79, p<0.01), dry gluten (r = 0.67, p<0.01) contents and the gluten index (r = 0.62, p<0.01), and negatively correlated with damage starch content (r = -0.23, p<0.05).

Table 3.8. Rheometric measurements of dough prepared from twenty-five wheat cultivars. Data represent the mean values ± standard deviation (n = 3).

Cultivar	Wheat Class	tan δ (-)	|G*| (kPa)	J_{max} (mPa^{-1})	J_{el} (-)
Harvest	CNHR	0.37 ± 0.02^{bcdefgh}	10.3 ± 2.7^{abc}	1.84 ± 0.79^{bcd}	0.62 ± 0.07^{a}
Lillian	CNHR	0.42 ± 0.02^{i}	10.8 ± 2.7^{abc}	2.37 ± 0.96^{cd}	0.57 ± 0.07^{a}
McKenzie	CNHR	0.36 ± 0.01^{abcdefgh}	13.4 ± 0.9^{abcde}	1.03 ± 0. 10^{ab}	0.63 ± 0.04^{a}
Pembina	CNHR	0.37 ± 0.00^{abcdefgh}	14.7 ± 0.8^{bcdef}	0.78 ± 0.03^{ab}	0.71 ± 0.03^{a}
Unity VB	CNHR	0.38 ± 0.03^{defgh}	13.0 ± 2.5^{abcde}	1.27 ± 0.67^{abc}	0.68 ± 0.15^{a}
5702PR	CPSR	0.33 ± 0.01^{ab}	18.8 ± 1.7^{f}	0.57 ± 0.09^{a}	0.69 ± 0.01^{a}
CDC Terrain	CPSR	0.35 ± 0.00^{abcde}	16.4 ± 0.9^{def}	0.64 ± 0.10^{ab}	0.68 ± 0.05^{a}
CDC Walrus	CWES	0.35 ± 0.00^{abcd}	13.8 ± 2.2^{abcde}	0.76 ± 0.24^{ab}	0.64 ± 0.06^{a}
AAC Iceberg	CWHWS	0.38 ± 0.01^{defgh}	11.1 ± 1.5^{abc}	1.31 ± 0.55^{abc}	0.71 ± 0.07^{a}
CDC Whitewood	CWHWS	0.36 ± 0.02^{abcdef}	12.8 ± 2.4^{abcd}	0.99 ± 0.37^{ab}	0.67 ± 0.04^{a}
Whitehawk	CWHWS	0.33 ± 0.00^{abc}	13.9 ± 0.8^{abcdef}	0.77 ± 0.11^{ab}	0.70 ± 0.03^{a}
AAC Brandon	CWRS	0.38 ± 0.01^{defgh}	12.0 ± 1.8^{abcd}	1.41 ± 0.45^{abcd}	0.52 ± 0.25^{a}
AAC Redwater	CWRS	0.38 ± 0.02^{defghi}	9.5 ± 1.6^{a}	1.82 ± 0.67^{abcd}	0.64 ± 0.02^{a}
Carberry	**CWRS**	**0.38 ± 0.01^{defgh}**	**12.0 ± 1.3^{abcd}**	**1.32 ± 0.28^{abc}**	**0.66 ± 0.03^{a}**
CDC Plentiful	CWRS	0.37 ± 0.00^{defgh}	11.7 ± 0.9^{abcd}	1.01 ± 0.05^{ab}	0.71 ± 0.01^{a}
CDC Stanley	CWRS	0.37 ± 0.01^{defgh}	12.0 ± 0.4^{abcd}	1.04 ± 0.12^{ab}	0.69 ± 0.01^{a}
CDC Titanium	CWRS	0.39 ± 0.01^{fghi}	10.8 ± 1.0^{abc}	1.45 ± 0.20^{abcd}	0.62 ± 0.04^{a}
CDC Utmost	CWRS	0.40 ± 0.01^{hi}	10.0 ± 1.8^{ab}	1.75 ± 0.49^{abcd}	0.66 ± 0.03^{a}
Glenn	**CWRS**	**0.33 ± 0.00^{a}**	**15.1 ± 1.8^{cdef}**	**0.64 ± 0.05^{ab}**	**0.68 ± 0.01^{a}**
Parata	CWRS	0.37 ± 0.00^{abcdefgh}	10.5 ± 1.2^{abc}	1.37 ± 0.29^{abcd}	0.65 ± 0.02^{a}
Roblin	CWRS	0.39 ± 0.01^{efghi}	9.7 ± 1.3^{a}	1.78 ± 0.30^{abcd}	0.50 ± 0.03^{a}
Shaw VB	CWRS	0.37 ± 0.00^{cdefgh}	9.2 ± 0.8^{a}	1.69 ± 0.34^{abcd}	0.66 ± 0.03^{a}
Stettler	CWRS	0.40 ± 0.01^{ghi}	9.6 ± 0.4^{a}	2.60 ± 0.19^{d}	0.62 ± 0.04^{a}
CDC Kinley	CWSP	0.36 ± 0.01^{abcdefg}	15.1 ± 1.0^{cdef}	0.77 ± 0.19^{ab}	0.66 ± 0.03^{a}
Pasteur	CWSP	0.36 ± 0.00^{abcdefgh}	17.9 ± 1.3^{ef}	0.59 ± 0.02^{ab}	0.69 ± 0.03^{a}
Average	**-**	**0.37**	**12.6**	**1.26**	**0.65**
Min/Max	**-**	**0.33/0.42**	**9.2/18.8**	**0.57/2.60**	**0.50/0.71**

Abbreviations: loss tangent (tan δ); complex modulus (|G*|); maximum creep compliance (J_{max}); and the relative elasticity (J_{el}). Data within the same colunm with different letters are significantly different (p<0.05).

In contrast, J_{el} was found to be negatively correlated with protein (r = -0.36, p<0.01), wet gluten (r = -0.35, p<0.01) and dry gluten (r = -0.30, p<0.01) contents, and the gluten index (r = - 0.26, p<0.01). In addition, Carberry and Glenn ranged from 0.38 to 0.33 for tan δ, and 12.1 to 15.1 kPa for |G*|, respectively. This represents that dough strength considered to be acceptable as CWRS cultivars may range within the values for the two cultivars.

For instance, the PCA found Whitehawk, CDC Kinley, CDC Walrus, Pembina, 5702PR, Pasteur, McKenzie, and CDC Terrain, to have similar dough strength to Glenn, based on shear rheology and creep compliance. In contrast, cultivars AAC Iceberg, Harvest, Parata, AAC Redwater, AAC Brandon, Shaw VB, CDC Utmost, Lillian and CDC Titanium were closely related to Carberry, closer to the weakest cultivars. Whereas Stettler, Roblin, CDC Plentiful, CDC Whitewood, and CDC Stanley were distinctly different from one another (Figure A3.6, Appendix).

3.5. Conclusion

Wheat flour quality for the diverse end-product uses is strongly influenced by the gluten composition and properties, which can be affected by the genotype, environment, and crop management practices. Specific wheat classes such as CWRS deliver consistent and desirable wheat quality for breadmaking. Therefore, the inter-relationship of wheat cultivars among different classes is crucial to determine wheat flour performance. For instance, Carberry and Glenn are commonly used as cultivar checks for breeding programs for new cultivars in the CWRS wheat class. Within the cultivar range used for the present study from 2016's crop year, clusters were formed around those two specific cultivars, which made it possible to classify and define wheat strength and dough handling characteristics. Overall, cultivar had a positive effect on proximate analysis and directly impacted dough handling parameters. In particular protein and gluten properties significantly (p<0.05) influenced the dough strength parameters. A significant correlation was found between gluten properties and dough strength measurements (p<0.01), such as micro-doughLAB and rheology. Wheat cultivars with high gluten strength, conferred stronger viscoelastic characteristics in both shear rheometry and creep compliance tests.

3.6. Linkage

Findings of this study described wheat grain, flour composition and dough handling results obtained for the 25 set of cultivars. From those, five cultivars were selected to be further

analyzed with the addition of enzymes and chemical additives. The decision was based on their protein content, gluten characteristics, and dough rheology performance. For instance, Glenn (1) is considered the higher end cultivar check for CWRS on dough strength and baking performance in the variety registration tests. Thus, it would set a high parameter for performance comparison either in dough handling, as well as, in baking. Stettler (2) was within the five most grown cultivars in Western Canada in 2016, however, it is known to have poor baking performance overall. The additives could be an alternative to improve its overall quality and acceptability in the baking industry. In addition, Stettler still considered CWRS cultivar by CGC. CDC Plentiful (3), in the other hand, has been performing well as a baking check within other varieties and LNT baking method, based on bread score and overall mixing time and dough handling. Therefore, both cultivars, Stettler and CDC Plentiful (remaining CWRS cultivars) would be suitable for intermediate strength of comparison. Cultivars Harvest (4) and Lillian (5) were two of the many cultivars that were moved from CWRS to CNHR class as proposed by the CGC. Therefore, there was an interest of further studying these cultivars effects towards enzymes and chemical oxidizers as being the weakest cultivars. The focus on the next study was to investigate the rheological behavior of each cultivar in relation to the addition of chemical oxidizers and enzymes in different concentrations, comparing them among each other and controls.

4. EFFECT OF CHEMICAL OXIDIZERS AND ENZYMATIC TREATMENTS ON THE RHEOLOGY OF DOUGH PREPARED FROM FIVE DIFFERENT WHEAT CULTIVARS[1]

4.1. Abstract

The use of enzymes is attractive to the baking industry as an alternative to chemical oxidizers, as dough strengtheners, resulting in cleaner label products (i.e., fewer ingredients). The quality parameters (proximate analysis, flour yield, gluten properties) and dough strength (i.e., empirical and fundamental rheology) of different wheat cultivars ranging in gluten strengths from weak (Harvest), intermediate (Lillian, CDC Plentiful and Stettler) to strong (Glenn) were analyzed with the addition of chemical oxidizers (i.e., ascorbic acid, azodicarbonamide) or commercial enzymes (i.e., glucose oxidase and fungal xylanase). Glenn showed better quality attributes compared to the other cultivars, and responded well to additives, especially glucose oxidase which significantly improved dough strength. Glucose oxidase also improved the dough handling of weaker cultivars. Overall, the addition of enzymes showed similar rheological behavior to dough prepared with chemical oxidizers.

Significance and novelty: The effect of enzymes in dough rheology and handling properties using fundamental and empirical rheology methods. In addition, a better understanding of the use of xylanase and its effect in different wheat strength (weak, intermediate, and strong).

4.2. Introduction

In Canada, wheat is one of the major crops produced with ~95% being grown across the Prairie Provinces. Because of its high quality, Canadian wheat has a high export demand by countries needing to improve the baking properties of their flour products. Canadian hard red spring is the largest market class grown in Canada. Hard red spring wheat is characterized by higher protein concentrations (12.8-14.8%), good processing characteristics, high-volume

[1] Tozatti, P., Hopkins, E.J., Briggs, C., Pierre Hucl, P., and Nickerson, M.T. (2019). Effect of chemical oxidizers and enzymatic treatments on dough rheology. *Journal of Cereal Science*, 90, 14 July 2019: 102806. https://doi.org/10.1016/j.jcs.2019.102806.

breads and the ability to improve the strength of low-protein wheat flours. In addition, hard red spring cultivars have good milling performance, ash content and brightness, and are generally resistant to pre-harvest sprouting (Dexter et al., 2006). Hard red spring wheat quality is largely dependent on its protein (gluten) content and composition, which is responsible for the viscoelastic properties of the dough and directly influence the final product characteristics (e.g., bread crumb structure and volume) (Joye et al., 2009). Furthermore, the glutenin polymer structure, size distribution and subunit composition, and the gliadin/glutenin ratio directly relate to gluten quality (Joye et al., 2009).

To overcome deficiencies in wheat quality, exogenous components can be incorporated to alter the functionality of the gluten proteins as a means to improve bread properties. These additives include chemical oxidizers, such as azodicarbonamide, ascorbic acid and peroxides (Joye et al., 2009). Oxidizing agents target the SH and S−S groups within the gluten to alter the strength of the network and its resulting viscoelasticity (Joye et al., 2009). Despite their performance in baking, concerns have been raised related to the consumption of chemical oxidizers, especially when they are attributed with precursors of cancer. For example, during baking, azodicarbonamide (ADA) forms biurea, semicarbazide (SEM) and urazole. ADA itself might cause allergic reactions and SEM has been linked to the development of some forms of cancer (Hirakawa et al., 2003; Bhagan et al., 2016). In addition, other studies reported that oral administration of semicarbazide results in angiomas, angiosarcomas and lung cancer in mice (Toth, 2000). The use of enzymes is attractive to industry since enzymes are considered processing aids and are not required to be put on the food package, creating a 'cleaner label' for the consumer. More recently, enzymes which favor protein crosslinking (e.g., transglutaminase, glucose oxidase, hexose oxidase and laccase) have been studied as an alternative strategy for strengthening the bread dough and improving bread quality (Joye et al., 2009). As an example, glucose oxidase (E.C. 1.1.3.4) (Gox) is commonly used as an alternative to chemical oxidizer agents in breadmaking. This enzyme catalyzes the reaction during dough kneading inducing the formation of disulphide bonds in gluten proteins (Stoica et al., 2009). The crosslinks are induced by coupling two cysteine residues within a food protein matrix, resulting in improved dough viscoelasticity and structural properties (Bonet et al., 2006).

The non-starch polysaccharides (NSP) in wheat are mainly composed of cellulose, arabinoxylan (AX), β-D-glucan and arabinogalactan. Up to 75% of the dry matter weight of wheat endosperm cell walls are composed of NSP, where the predominant group is AX (~85%). However, the amount, structure and functional properties can vary according to the wheat cultivar (i.e., molecular weight and distribution, branching pattern, extractability with water,

interaction with other cell wall components such as lignin or cellulose). Non-starch polysaccharides are also closely related to gluten forming proteins and clearly modify their properties, influencing the functional and dough rheological properties (Goesaert et al., 2005). Arabinoxylans originate from the aleurone and bran layers of the wheat kernel and significantly increase the water holding capacity in flour (Kweon et al., 2011). The water-extractable arabinoxylans have higher water holding capacity than damaged starch or gluten does, which results in a beneficial effect on baking (Kweon et al., 2011). In baking, xylanase hydrolyzes both soluble and insoluble pentosans in the flour, which in turn leads to better water absorption. As a result, dough handling is improved, along with dough stability, crumb structure and loaf volume (Ahmad et al., 2014). This enzyme acts during the gluten re-agglomeration which happens after the breakdown of gluten structures during mixing, affecting gluten yield and gluten rheological properties.

The overall goal of this study was to examine the effect of various chemical oxidizers (ADA, ascorbic acid) and commercial enzymes (glucose oxidase and fungal xylanase) on the rheology of dough prepared using five different commercially grown hard red spring cultivars, representing a range of gluten strengths (weak to strong). Although studies exist in the literature looking at different additives on dough properties, few combine both chemical and enzymatic additives in the same study, nor consider their impact on cultivars that range in strengths.

4.3. Materials and methods

4.3.1. Materials

Five red hard spring wheat cultivars were grown in a uniform unreplicated seed multiplication nursery in 2017 at the University of Saskatchewan's Kernen Crop Research Farm (Saskatoon, SK). These were Glenn, Harvest, Lillian, CDC Plentiful and Stettler. These cultivars were selected to have weak (Harvest), intermediate (Lillian, CDC Plentiful and Stettler) and strong (Glenn) gluten strengths and dough rheology. The chemicals ascorbic acid and azodicarbonamide were purchased from Sigma-Aldrich Co. (Oakville, ON, Canada). Enzymes, including glucose oxidase (Grindamyl S758) and fungal xylanase (Grindamyl S250), were kindly donated by DuPont (DuPont Nutrition and Health: New Century, KS, U.S.A.).

4.3.2. Flour preparation

Prior to milling, seeds from each cultivar (~5 g) were ground, using a Thomas–Wiley laboratory grinder (model 4, Arthur H. Thomas Co.: Philadelphia, PA, USA), into meal for moisture determination, which was performed according to AACCI Approved Method 44-

15.02. Based on the determined seed moisture, all wheat cultivars were tempered to 14.5% moisture for ~18 h, and then milled into flour on a Brabender Quadrumat Senior Experimental Mill (Brabender: South Hackensack, NJ, USA), as modified by Jeffers and Rubenthaler (1977). Milling was performed at the University of Saskatchewan in the Grains Innovation Laboratory. Data represents the mean ± one standard deviation (n = 3).

4.3.3. Flour composition and quality

Crude protein (% N x 5.7), moisture and ash content of all flours were measured according to AACCI Approved Methods 4630.01, 4415.02 and 0803.01, respectively. Crude lipid content was measured for all flours using an ANKOM Extraction System XT-15 (ANKOM Technology, NY, USA) following AOCS Standard Procedure Am 5-04 (AOCS, 2005). Grain hardness index (Single Kernel Characterization System – SKCS4100) and Falling Number were determined using AACCI Approved Methods 55-31.01 and 56-81.03, respectively. Gluten properties (gluten index, wet/dry gluten content) were assessed using the Glutomatic System (Perten Instruments, Sweden), according to AACCI Approved Method 38-12.02. The damaged starch content in the flours was determined with a Megazyme assay kit (Megazyme International, Bray, Ireland) according to the AACCI Approved Method 76-31.01. Data represents the mean ± one standard deviation (n = 3).

4.3.4. Solvent retention capacity

The solvent retention capacity (SRC) test was performed based on the AACCI Approved Method 56-11.02, with modifications (Kweon et al., 2011). In brief, different solvents were initially prepared, including: (1) deionized and distilled water (DDH_2O) *(i.e., associated with all components*); (2) 50% sucrose solution (w/w) (*i.e., associated with pentosan components*); (3) 5% sodium carbonate solution (w/w) (*i.e., associated with levels of damaged starch*); and (4) 5% lactic acid solution (w/w) (i.*e., associated with glutenin proteins)*. Approximately 5 g of each flour was weighed into 4 x 50 mL conical bottom polypropylene centrifuge tubes (pre-weighed with top lid), followed by the addition of 25 g of each solvent (i.e., solvents 1, 2, 3 and 4). Each tube was vigorously mixed for 5 s at 5, 10, 15, and 20 min. The samples were centrifuged for 15 min at 1000 × g (VWR Clinical 200, Mississauga, ON, Canada). After centrifugation, the tubes was drained and inverted for 10 min, and the remaining residue was weighed. The % SRC for each solvent was calculated based on Eq. 4.1. The gluten performance index (GPI) was determined from the % SRC percent for lactic acid, sodium carbonate, and sucrose – as shown in Eq. 4.2. (Kweon et al., 2011).

$$\% SRC = \left[\frac{gel\ weight}{flour\ weight} - 1\right] * \left[\frac{86}{100 - \% flour\ moisture}\right] * 100 \quad \text{(Eq. 4.1)}$$

$$GPI = \frac{Lactic\ acid\ SRC}{(sodium\ carbonate\ SRC + sucrose\ SRC)} \quad \text{(Eq. 4.2)}$$

The GPI is a predictive parameter used to describe the overall performance of glutenin while in the network system of the other wheat flour polymers (Kweon et al., 2011; Hammed et al., 2015). Data represents the mean ± one standard deviation (n = 3).

4.3.5. Micro-doughLAB and Mixograph

Flour (~4 g) for each cultivar was weighed based on a 14% m.b. (moisture basis). The samples were mixed at a constant speed of 63 rpm using a Newport Micro-doughLAB mixer (Perten Instruments, Sweden). The amount of water added was determined as the water needed to achieve a dough consistency of 500 BU. The water absorption (ABS), dough development time (DDT), stability (STA), and mixing tolerance index (MTI) were calculated using DLW version 1.0.0.56 software. Data represents the mean ± one standard deviation (n = 3). Dough mixing properties were also measured using the Mixograph, (TMCO National Mgf, Lincoln, NE), according to AACCI Approved Method 54-40.02. The optimum dough water absorption (14% m.b.) was estimated based on the protein content of the flour, using the following formula (as per the method):

$$Y = 1.5X + 43.6 \quad \text{(Eq. 4.3)}$$

where *X* is% flour protein content (14% m.b.) and *Y* is % of water absorption. The flour and water were then added to a 10 g mixograph bowl, and then allowed to mix for 10 min. Parameters such as mixograph development time, peak dough resistance, energy to peak, and bandwidth at peak were determined. Data represents the mean ± one standard deviation (n = 3).

4.3.6. Rheology: Small amplitude shear rheometry

Preparation of dough

Dough for rheology analysis was prepared using a 10 g Mixograph (TMCO National Mfg., Lincoln, NE) following AACCI Approved Method 54-40.02. The basic formulation

included flour (weight on a 14% m.b.), water (weight based on micro-doughLAB absorption results) and NaCl (2.0 % by weight). Chemical oxidizers were added at two concentrations, representing 50% and 100% of the allowable limits for the baking industry from Health Canada (2012). For ascorbic acid and azodicarbonamide, these limits are 200 and 45 ppm of flour, respectively (Health Canada, 2012). Glucose oxidase and fungal xylanase were also used at two concentrations, representing 50 and 100% of the maximum recommended concentration by the company; which was 150 and 300 ppm respectively (DuPont, New Century, KS, USA). All additives were added to the water phase prior to mixing with the other ingredients to ensure homogeneity of the sample. Doughs were prepared at a constant moisture content based on micro-doughLAB absorption for each cultivar and mixed to peak dough development. All prepared doughs were allowed to rest for 60 min in a sealed container (to prevent drying) at room temperature, before performing the rheological testing to allow for enzyme activity to proceed. Dough without chemical oxidizers or enzymes for each cultivar served as a control to these treatments. Doughs for each treatment were prepared in triplicate.

Rheology

Rheological tests on doughs were completed with an AR-2000 rheometer (TA Instruments, New Castle, DE, USA) utilizing a 40 mm parallel plate fixture, based on the method of Jekle and Becker (2011). In brief, a dough sample (~5 g) was placed between the plates and once the plate was lowered the excess was removed. Paraffin oil was added to the surface of the dough (i.e., to ensure that the dough would not dry out). Before testing, the dough rested for approximately 10 min. During the analysis, the gap was set at 2 mm, and the temperature was kept constant at room temperature (21-23°C) until the end of the experiment. The dynamic storage (G′), loss (G″), and complex moduli (|G*|) and loss tangent (tan δ) were determined as a function of frequency (0.1-100 Hz) at a constant amplitude strain of 0.1%. Values at a frequency of 1 Hz were arbitrarily selected for comparative purposes between cultivars. After the oscillatory frequency test, creep recovery was performed on the same dough sample at a constant shear stress (τ_0 = 250 Pa) for 180 s. After this applied stress, stress was removed and the relaxation of the dough was observed for 360 s. As a function of time, the strain values were recorded and evaluated with the following equation:

$$J(t) = \gamma(t)\tau_0^{-1} \quad \text{(Eq. 4.4)}$$

where J is the compliance (Pa^{-1}), t is the time (s), γ is the strain, and τ_0 is the stress (constant) applied during test. The creep compliance J_{max} (at t = 180 s of the creep phase) and J_r (t= 360 s in the recovery phase) were used to determine the relative elasticity of the sample (J_{el}) by the following equation (Eq. 5):

$$J_{el} = J_r(J_{max})^{-1} \quad \text{(Eq. 4.5)}$$

All oscillatory rheology measurements were made within the linear viscoelastic regime, however the creep compliance was not. Two measurements were performed on each of the three biological replicates (n = 3). Data represents the mean ± one standard deviation.

4.3.7. Statistics

A one-way analysis of variance (ANOVA) and a Tukey Post-Hoc test were performed to determine the statistical differences for each cultivar for their compositional properties, quality attributes, solvent retention capacities, and micro-doughLAB and mixograph results. *P-values* <0.05 were considered statistically different. Principal component analysis was performed on each of the compositional, solvent retention, micro-doughLab, mixograph and rheological data. All statistical analysis was performed using SPSS Grad Pack v24 software (IBM Corp, Armonk, NY).

4.4. Results and discussion

4.4.1 Flour composition and quality

Analysis of flour composition of the five wheat cultivars at 14% m.b. is presented in Table 4.1. Protein content was the lowest for CDC Plentiful (12.1%), followed by Harvest and Lillian (13.2%), and then Glenn and Stettler (~14.0%) (p<0.05). Ash and lipid levels were all found to be similar at 0.32% and 0.7%, respectively, across all examined cultivars (p>0.05). In contrast, the damaged starch content was 4.2% on average; Glenn contained the lowest levels of damaged starch (3.4%), followed by Lillian (3.9%), and then Harvest, Stettler and CDC Plentiful which contained the highest levels, and were similar at 4.7% (p<0.05).

Table 4.1. Composition (14% m.b.), quality and solvent retention capacity of flours from five Canadian hard red spring wheat cultivars. Data represents the mean of replicate measurements ± one standard deviation (n=3).

Property	Wheat cultivars					Min/Max	Avg.	Target range[3]
	Harvest	Lillian	Glenn	Stettler	CDC Plentiful			
Composition[1]								
Moisture, %	14.9 ± 0.0^{c}	15.0 ± 0.0^{c}	14.5 ± 0.0^{b}	15.0 ± 0.0^{c}	13.9 ± 0.1^{a}	13.9/15.0	14.7	14.0
Protein, %	13.1 ± 0.1^{b}	13.4 ± 0.2^{b}	14.0 ± 0.4^{c}	14.1 ± 0.2^{c}	12.1 ± 0.1^{a}	12.1/14.1	13.3	12.8
Ash, %	0.33 ± 0.00^{a}	0.38 ± 0.09^{a}	0.29 ± 0.02^{a}	0.31 ± 0.00^{a}	0.31 ± 0.03^{a}	0.31/0.38	0.32	0.42
Lipids, %	0.7 ± 0.2^{a}	0.8 ± 0.3^{a}	0.7 ± 0.2^{a}	0.9 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4/0.9	0.7	-
Damaged starch, %	4.6 ± 0.3^{b}	3.9 ± 0.4^{ab}	3.4 ± 0.6^{a}	4.6 ± 0.3^{b}	4.7 ± 0.2^{b}	3.4/4.7	4.2	6.1
Quality parameters[1]								
Flour Yield, (%)	72.3 ± 0.2^{b}	71.2 ± 0.4^{a}	71.8 ± 0.6^{ab}	72.1 ± 0.4^{ab}	72.7 ± 0.4^{b}	71.2/72.7	72.0	76
SKCS-HI	81 ± 2^{d}	68 ± 1^{a}	78 ± 1^{cd}	71 ± 3^{ab}	74 ± 1^{bc}	68/81	74	-
Falling number, (s)	394 ± 2^{c}	417 ± 9^{d}	317 ± 1^{a}	384 ± 2^{bc}	382 ± 2^{b}	317/417	379	400
Wet gluten, %	40.6 ± 0.6^{b}	40.3 ± 0.8^{b}	35.4 ± 0.5^{a}	43.2 ± 0.5^{c}	36.8 ± 0.3^{a}	35.4/43.2	39.3	33.9
Dry gluten, %	14.3 ± 0.4^{b}	14.0 ± 0.2^{ab}	13.2 ± 0.3^{a}	15.3 ± 0.4^{c}	13.1 ± 0.5^{a}	13.1 /14.3	14.0	-
Gluten index, %	84.7 ± 1.1^{b}	78.1 ± 3.0^{a}	98.4 ± 0.4^{d}	90.6 ± 1.8^{c}	96.5 ± 1.5^{d}	78.1/98.4	89.7	94.1
Solvent retention capacity[2]								
50% (w/w) Sucrose	96.4 ± 0.5^{a}	108.8 ± 0.7^{c}	103.1 ± 1.9^{b}	100.9 ± 0.8^{b}	108.4 ± 0.7^{c}	96.4/108.8	103.5	105-115
5% (w/w) Lactic acid	144.6 ± 1.2^{a}	159.9 ± 1.9^{c}	165.3 ± 0.6^{d}	151.6 ± 1.3^{b}	167.2 ± 1.3^{d}	144.6/167.2	157.7	>140
5% (w/w) Sodium carbonate	74.1 ± 0.8^{ab}	80.6 ± 0.6^{c}	76.8 ± 1.6^{b}	72.4 ± 0.5^{a}	79.8 ± 1.1^{c}	72.4/80.6	76.7	80-90
ddH_20	63.5 ± 1.2^{ab}	66.0 ± 0.8^{b}	65.3 ± 1.7^{b}	62.0 ± 0.1^{a}	65.2 ± 0.9^{b}	62.0/66.0	64.4	65-70
Gluten performance index	0.85 ± 0.01^{ab}	0.84 ± 0.01^{a}	0.92 ± 0.02^{c}	0.88 ± 0.01^{b}	0.89 ± 0.02^{b}	0.84/0.92	0.88	0.75

[1]Based on 14%, m.b.

[2]Solvent retention capacity solutions relate to levels of pentosans (50% w/w sucrose), gluten proteins (5% lactic acid), damaged starch (5% sodium carbonate) and all components (ddH_20).

[3]Target ranges were provided by www.uswheat.org and Canadian Grain Commission (CGC, 2018b).

Data within the same row with different letters are significantly different ($p<0.05$).

Lillian and Stettler had the lowest SKCS-hardness indices (68 and 71, respectively), followed by CDC Plentiful (74), and then Glenn (78) and Harvest (81) (p<0.05). Falling numbers (FN) were the lowest in the case of Glenn (317 s), followed by Stettler and CDC Plentiful (383 s), whereas Harvest (394 s) and Lillian (417 s) were the highest (p<0.05) (Table 4.1). All flours showed low enzymatic activity, with an average FN of 379 s indicating good sound grain. In the case of the gluten index (GI), Lillian had the lowest value (78%), followed by Harvest (85%), and Stettler (91%), then Glenn and CDC Plentiful which were similar at 97% (p<0.05) (Table 4.1). Results indicated that the gluten proteins were strongest in Glenn and CDC Plentiful, and weakest in Lillian. The water binding properties of the gluten proteins are described by the wet gluten content, where Glenn and CDC Plentiful were both found to have the lowest values (36.1%), followed by Harvest and Lillian (40.4%) and then Stettler (43.2%) (p<0.05) (Table 4.1). The dry gluten was lowest for CDC Plentiful and Glenn (13.1%), followed by Lillian and Harvest (14.2%), and then Stettler (15.3%) (p<0.05) (Table 4.1).

The solvent retention capacity (SRC) provides an indication of the relative compatibility of different flour components, such as gluten proteins, damaged starch and arabinoxylans (pentosans), in different solvents. This compatibility can be related in terms of their abilities to absorb water, being a well correlated method for milling, breadmaking, and baking quality parameters prediction (Hammed et al., 2015). Table 4.1 shows SRC values within various solvents. Based on the results for distilled, deionized water, which represents the absorption properties of all components in the flour, Stettler and Harvest had the lowest absorption capacity with (62.5), followed by Lillian, Glenn and CDC Plentiful which had values averaging 65.5 (p<0.05). In terms of the contributions of arabinoxylans (50% w/w sucrose) to water absorption, Harvest had the least water absorption from this component (96), whereas it was increased with Glenn and Stettler (102), and highest with Lillian and CDC Plentiful (108) (p<0.05). Contributions from gluten proteins (examined with the 5% lactic acid solvent) to water absorption was lowest from Harvest (145), and increased in absorption with Stettler (152) Lillian (160) and then Glenn and CDC Plentiful, which had the highest absorption (166) (p<0.05).

These values did not correlate well to the wet gluten content in the flour, where Glenn and CDC Plentiful had the least amount of wet gluten present. Contributions from damaged starch (examined with the 5% sodium carbonate solvent) to water absorption was lowest for Stettler and Harvest (73), followed by Glenn (77) and then Lillian and CDC Plentiful (80) (p<0.05) (Table 4.1). This trend was unexpected because damaged starch generally increases water absorption and Harvest had the highest concentration of damaged starch (4.6%) and

Lillian and Glenn had the least damaged starch (Goesaert et al., 2005). The gluten performance index (GPI) is an indicator of the overall performance (e.g., gluten network and baking) of the glutenin subunits in the flour (Kweon et al., 2011). GPI was lowest for Harvest and Lillian (0.84), followed by Stettler and CDC Plentiful (0.88) and then Glenn (0.92) (p<0.05). The contributions from pentosans and gluten proteins to water absorption, and the GPI for all five flours were all above or within the industry range for a high-quality baking flour (0.75 – 0.88). Similar results for hard red spring wheat flours were reported by Hammed et al., (2015), with GPI values ranging from 0.63 to 0.78. In contrast, contributions from damaged starch and the entire flour components to water absorption were both slightly lower than the industry standard (Table 4.1). Principle component analysis of the compositional and solvent retention capacity data revealed that all cultivars were distinctly different from one another (Figure A4.1 and A4.2, Appendix).

4.4.2. Dough mixing behavior for each wheat cultivar

Dough mixing behavior for dough prepared using the five cultivars was examined using the mixograph and the micro-doughLAB, and are presented in Table 4.2. Although both techniques measure similar characteristics of the dough, they are not directly comparable. For the mixograph data, mixograph development time (MDT) or the mixing time to peak was similar for the five cultivars at 2.9 min, except for Glenn which was significantly higher (4.3 min) (p<0.05). MDT values give an indication of the optimum mixing time in order for dough to reach maximum consistency, which is important for understanding gluten strength and can give an indication of breadmaking quality (Ohm and Chung, 2018). Peak dough resistance (PDR), which relates to the percentage of torque at peak development, was lowest in the case of Lillian (51.1% torque), followed by Glenn and Harvest (57.8% torque), and then Stettler and CDC Plentiful (62.9% torque), which had the highest PDR (p<0.05). Typically, stronger flours are likely to have higher PDR values (Wooding et al., 1999). Bandwidth at peak resistance (BPR), which refers to the percentage of the torque at peak dough resistance, was lowest for Lillian and Stettler (27.7% torque), followed by Harvest and Glenn (35.6% torque), and then CDC Plentiful (40.0% torque) (p<0.05). Work input to peak (WIP) describes the amount of energy needed to bring the dough to its optimal development for breadmaking (Wooding et al., 1999). Lillian and Harvest (105% tq.min) had the lowest WIP values, followed by CDC Plentiful (127% tq.min) and Glenn and Stettler (164 % tq.min) with the highest. Typically, stronger flours have longer MDTs and higher PDR, BPR and WIP values (Wooding et al., 1999).

Table 4.2. Dough mixing behavior and rheology of five Canadian hard red spring wheat cultivars. Data represents the mean of replicate measurements ± one standard deviation (n=3).

Property	Wheat cultivars						
	Harvest	**Lillian**	**Glenn**	**Stettler**	**CDC Plentiful**	**Min/Max**	**Avg.**
Mixograph							
Mixograph development time, min	2.8 ± 0.3^{a}	2.8 ± 0.1^{a}	4.3 ± 0.21^{b}	3.1 ± 0.2^{a}	2.9 ± 0.1^{a}	2.8/4.3	3.2
Peak dough resistance, % torque	58.6 ± 2.2^{b}	51.1 ± 1.5^{a}	56.8 ± 2.9^{b}	63.1 ± 2.0^{c}	62.6 ± 2.9^{c}	51.1/63.1	58.5
Bandwidth at peak resistance, %	34.7 ± 3.7^{bc}	25.2 ± 1.6^{a}	36.4 ± 5.2^{bc}	30.1 ± 3.3^{ab}	40.0 ± 2.3^{c}	25.2/40.0	33.3
Work input to peak , % tq.min	107 ± 8^{a}	102 ± 4^{a}	166 ± 3^{c}	158 ± 15^{bc}	127 ± 7^{ab}	102 / 166	132
Micro-doughLAB							
Absorption (ABS), %	60.7 ± 0.4^{c}	61.2 ± 0.1^{c}	58.0 ± 0.3^{a}	59.8 ± 0.3^{b}	57.8 ± 0.4^{a}	57.8/61.2	59.5
Stability (STA), min	5.5 ± 0.7^{a}	4.6 ± 0.1^{a}	10.2 ± 0.6^{c}	7.9 ± 0.3^{b}	8.2 ± 0.5^{b}	4.6/10.2	7.3
Dough Development time (DDT), min	4.2 ± 0.1^{ab}	3.3 ± 0.1^{a}	7.5 ± 0.5^{c}	4.2 ± 0.5^{ab}	4.9 ± 0.4^{b}	3.3/7.5	4.8
Mixing Tolerance Index (MTI)	53.3 ± 7.6^{b}	56.6 ± 2.9^{b}	41.6 ± 2.9^{a}	40.1 ± 0.0^{a}	41.6 ± 2.9^{a}	40.0/56.6	46.6

Data within the same row with different letters are significantly different ($p<0.05$).

However, the results from this study show no clear strongest dough cultivar based on a comparison of these parameters, as the trends are mixed. Principle component analysis of the mixograph data revealed that all cultivars were distinctly different from one another (Figure 4A.1, Appendix).

For the micro-doughLAB assessment, the water absorption, stability, dough development time and mixing tolerance index were measured. For breadmaking, flour absorption usually varies from 58-62% (Cauvain, 2015c). In the present study (Table 4.2), water absorption was lowest for Glenn and CDC Plentiful (58%), followed by Stettler (60%), and then Harvest and Lillian (61%) ($p<0.05$). The water absorption is influenced and dependent on different parameters, such as moisture content (i.e., higher moisture, less absorption), protein content (i.e., protein absorbs its own weight in water), wheat hardness, starch damage level and pentosan or arabinoxylans content (i.e., high water-binding capacity) and composition (Cauvain, 2015c; Sapirstein et al., 2018). In addition, water plays an important role in determining the viscoelastic properties of dough, reducing the dynamic properties proportionally, and as a lubricant, enhancing the relaxation. A strong correlation was found between water absorption (ABS) and GI and STA, ($r = 0.94$ and 0.88), respectively ($p<0.001$). Dough stability is the time difference between the point where the top of the curve first intercepts the 500 BU line and the point where the top of the curve leaves the 500 BU line. Stability (STA) was lowest for Lillian and Harvest, followed by Stettler and CDC Plentiful, and then Glenn ($p<0.05$). Typically, higher water absorption and longer stability characterize stronger wheat flours (Preston et al., 2001). In addition, the dough development time (DDT) average was 4.8 min, where it was shortest for Lillian, followed by Harvest, Stettler and CDC Plentiful, and then Glenn ($p<0.05$). Similar results were reported by Preston et al., (2001) when analyzing a range of high grade Canadian western red spring with STA of 10.1 min and DDT of 5.8 min, on average. Mixing tolerance index (MTI) was lowest for Glenn, Stettler, and CDC Plentiful, and higher for Harvest and Lillian ($p<0.05$). In addition, cultivars that required higher energy showed higher stability and DDT (Glenn, Stettler, and CDC Plentiful). Those characteristics are usually related to stronger wheat flours (Preston et al., 2001).

The overall mixing properties of Canadian western red spring can be strongly influenced by both cultivar and environment (Preston et al., 2001). In the present study (Table 4.2), Glenn performed as the strongest cultivar; it had a higher mixing time (4.3 min), stability (10.2 min), protein content (14.0%), and energy required to peak (166% tq.min) compared to the other cultivars assessed. In addition, Glenn had the highest dough stability (10.2 min) by a significant margin in comparison to all the other cultivars. Stronger wheat cultivars tend to

require more mixing time which usually results in more dough stability, and requires more energy to develop the dough to its peak (Malalgoda et al., 2018). Alternatively, Harvest and Lillian required the lowest energy to get to the peak dough development (107 and 102 %tq.min, respectively), they also had the lowest MTI (53.3 and 56.7, respectively). This indicates that these cultivars are weaker than the other examined cultivars, and possibly less suited to breadmaking than the others in the current study. Principle component analysis of the mixograph data found that Harvest and Lillian, Plentiful and Stettler, and Glenn were distinctly different from one another (Figure 4A.1D, Appendix).

4.4.3. Dough rheology in the presence of chemical oxidizers and enzymes

A rheometer was used to measure the complex shear modulus (|G*|) and loss tangent (tan δ) in oscillatory shear mode, and the maximum compliance (J_{max}) and relative elasticity (J_{el}) in creep recovery mode, for dough prepared as a function of cultivar-type, additive and additive concentration, with results given in Table 4.3. A 3-way ANOVA was performed for |G*| tan δ, J_{el} and J_{max} for all formulations. In the case of |G*|, all main effects, 2-way interactions and the 3-way interaction were significant (Table A4.1, Appendix). Similar findings were found for tan δ, except the main effect of additive concentration and the 2-way interaction between cultivar × additive concentration were not significant. In contrast, no significant differences were reported between treatments for either J_{max} or J_{el}.

Table 4.3. Dough rheology of five Canadian hard red spring wheat cultivars measured with different additives (chemical oxidizers and enzymes). Data represents the mean of replicate measurements ± one standard deviation (n=3).

Cultivar	Additive	Conc'n	tan δ (-)	|G*| (kPa)	J_{max} (mPa^{-1})	J_{el} (-)
Glenn	Null	-	0.32 ± 0.01^{bc}	19.1 ± 0.9^{a}	0.34 ± 0.05^{c}	0.75 ± 0.01^{a}
	AA	50%	0.30 ± 0.00^{ab}	22.1 ± 0.8^{abc}	0.27 ± 0.05^{abc}	0.74 ± 0.03^{a}
		100%	0.30 ± 0.01^{ab}	20.4 ± 0.6^{ab}	0.29 ± 0.03^{bc}	0.75 ± 0.01^{a}
	ADA	50%	0.31 ± 0.01^{abc}	25.9 ± 1.6^{cd}	0.23 ± 0.01^{ab}	0.73 ± 0.03^{a}
		100%	0.31 ± 0.00^{ab}	21.0 ± 1.1^{ab}	0.28 ± 0.04^{abc}	0.77 ± 0.01^{a}
	Gox	50%	0.29 ± 0.01^{a}	23.1 ± 1.1^{bcd}	0.22 ± 0.01^{ab}	0.77 ± 0.02^{a}
		100%	0.29 ± 0.01^{a}	26.3 ± 2.4^{d}	0.19 ± 0.04^{a}	0.76 ± 0.02^{a}
	Xyl	50%	0.33 ± 0.00^{bc}	21.4 ± 1.1^{ab}	0.32 ± 0.02^{c}	0.73 ± 0.00^{a}
		100%	0.33 ± 0.00^{c}	21.1 ± 1.8^{ab}	0.34 ± 0.02^{c}	0.72 ± 0.03^{a}
	Min		**0.29**	**19.1**	**0.19**	**0.72**
	Max		**0.33**	**26.3**	**0.34**	**0.77**

Table 4.3. (cont...)

Cultivar	Additive	Conc'n	tan δ (-)	|G*| (kPa)	J_{max} (mPa^{-1})	J_{el} (-)
Harvest	Null	-	0.33 ± 0.01^{bc}	13.7 ± 0.3^{ab}	0.99 ± 0.50^{c}	0.57 ± 0.23^{a}
	AA	50%	0.30 ± 0.00^{a}	17.7 ± 0.6^{cd}	0.45 ± 0.04^{ab}	0.64 ± 0.05^{a}
		100%	0.31 ± 0.01^{a}	14.7 ± 1.9^{abc}	0.49 ± 0.06^{abc}	0.71 ± 0.04^{a}
	ADA	50%	0.31 ± 0.00^{ab}	16.7 ± 1.0^{bcd}	0.41 ± 0.02^{a}	0.76 ± 0.01^{a}
		100%	0.31 ± 0.01^{ab}	15.5 ± 1.5^{bcd}	0.47 ± 0.08^{ab}	0.77 ± 0.00^{c}
	Gox	50%	0.30 ± 0.00^{a}	18.8 ± 0.5^{d}	0.36 ± 0.10^{a}	0.75 ± 0.02^{a}
		100%	0.30 ± 0.00^{a}	17.1 ± 2.1^{bcd}	0.30 ± 0.04^{a}	0.75 ± 0.01^{a}
	Xyl	50%	0.34 ± 0.01^{c}	15.3 ± 0.7^{abc}	0.56 ± 0.07^{abc}	0.71 ± 0.01^{a}
		100%	0.36 ± 0.01^{d}	12.0 ± 0.5^{a}	0.94 ± 0.09^{bc}	0.71 ± 0.01^{a}
	Min		**0.30**	**12.0**	**0.30**	**0.57**
	Max		**0.36**	**18.8**	**0.94**	**0.77**
Lillian						
	Null	-	0.35 ± 0.01^{b}	17.6 ± 1.4^{ab}	0.51 ± 0.08^{a}	0.72 ± 0.01^{a}
	AA	50%	0.34 ± 0.00^{ab}	16.8 ± 0.5^{b}	0.46 ± 0.03^{a}	0.72 ± 0.03^{a}
		100%	0.32 ± 0.02^{ab}	19.6 ± 1.0^{b}	0.35 ± 0.04^{a}	0.73 ± 0.01^{a}
	ADA	50%	0.33 ± 0.00^{ab}	18.8 ± 1.4^{c}	0.35 ± 0.01^{a}	0.75 ± 0.01^{a}
		100%	0.34 ± 0.01^{b}	16.8 ± 0.9^{ab}	0.45 ± 0.02^{a}	0.78 ± 0.04^{a}
	Gox	50%	0.33 ± 0.01^{ab}	20.4 ± 0.8^{b}	0.33 ± 0.03^{a}	0.74 ± 0.01^{a}
		100%	0.31 ± 0.01^{a}	20.3 ± 2.7^{b}	0.29 ± 0.06^{a}	0.75 ± 0.00^{a}
	Xyl	50%	0.39 ± 0.01^{c}	14.1 ± 1.7^{a}	0.78 ± 0.02^{a}	0.71 ± 0.02^{a}
		100%	0.39 ± 0.00^{c}	13.7 ± 0.3^{a}	0.81 ± 0.06^{a}	0.71 ± 0.01^{a}
	Min		**0.31**	**13.7**	**0.29**	**0.71**
	Max		**0.39**	**20.4**	**0.81**	**0.78**
CDC Plentiful						
	Null	-	0.34 ± 0.01^{bc}	20.3 ± 1.1^{cd}	0.37 ± 0.04^{ab}	0.72 ± 0.02^{a}
	AA	50%	0.32 ± 0.00^{ab}	19.5 ± 1.3^{cd}	0.33 ± 0.03^{ab}	0.74 ± 0.02^{a}
		100%	0.31 ± 0.00^{a}	22.6 ± 2.0^{d}	0.28 ± 0.02^{a}	0.74 ± 0.02^{a}
	ADA	50%	0.33 ± 0.00^{abc}	20.6 ± 1.5^{cd}	0.36 ± 0.03^{ab}	0.73 ± 0.02^{a}
		100%	0.34 ± 0.01^{c}	18.6 ± 0.9^{bc}	0.45 ± 0.03^{b}	0.74 ± 0.01^{a}
	Gox	50%	0.33 ± 0.01^{bc}	19.8 ± 1.4^{cd}	0.36 ± 0.06^{ab}	0.74 ± 0.03^{a}
		100%	0.32 ± 0.00^{ab}	19.1 ± 1.3^{cd}	0.32 ± 0.02^{ab}	0.76 ± 0.03^{a}
	Xyl	50%	0.36 ± 0.01^{d}	15.3 ± 1.7^{ab}	0.65 ± 0.13^{c}	0.72 ± 0.01^{a}
		100%	0.37 ± 0.00^{d}	14.1 ± 0.2^{a}	0.68 ± 0.04^{c}	0.74 ± 0.01^{a}
	Min		**0.31**	**14.1**	**0.28**	**0.72**
	Max		**0.37**	**22.6**	**0.68**	**0.76**

Table 4.3. (cont...)

Stettler						
	Null	-	0.35 ± 0.00^{c}	11.6 ± 0.6^{ab}	0.75 ± 0.07^{a}	0.73 ± 0.00^{a}
	AA	50%	0.34 ± 0.01^{abc}	14.1 ± 0.4^{de}	0.50 ± 0.02^{a}	0.77 ± 0.01^{a}
		100%	0.32 ± 0.01^{a}	16.0 ± 0.6^{d}	0.42 ± 0.04^{a}	0.75 ± 0.02^{a}
	ADA	50%	0.34 ± 0.01^{abc}	13.4 ± 0.5^{bc}	0.53 ± 0.06^{a}	0.78 ± 0.01^{a}
		100%	0.35 ± 0.00^{bc}	12.4 ± 0.6^{abc}	0.63 ± 0.06^{a}	0.77 ± 0.00^{a}
	Gox	50%	0.34 ± 0.01^{abc}	16.0 ± 1.0^{d}	0.44 ± 0.09^{a}	0.75 ± 0.02^{a}
		100%	0.33 ± 0.01^{ab}	14.3 ± 1.2^{cd}	0.44 ± 0.05^{a}	0.77 ± 0.01^{a}
	Xyl	50%	0.39 ± 0.01^{d}	11.2 ± 0.5^{a}	1.04 ± 0.18^{a}	0.70 ± 0.01^{a}
		100%	0.38 ± 0.01^{d}	13.1 ± 0.6^{abc}	1.78 ± 1.62^{a}	0.56 ± 0.32^{a}
	Min		**0.32**	**6.8**	**0.42**	**0.56**
	Max		**0.39**	**16.0**	**1.78**	**0.78**

Data within the same colunm with different letters are significantly different (p<0.05).

Figure 4.1 and 4.2 show the 3-way interaction for |G*| and tan δ, respectively. Findings were highly cultivar and additive specific. In the case of Glenn, no effect of additive concentration was seen for ascorbic acid (AA), fungal xylanase (Xyl), Gox or the control (null), however in the case of ADA stronger doughs were formed when 100% of the permitted level was used relative to when 50% was used (Figure 4.1A). Overall, doughs prepared with Gox were stronger than the others, with the exception of doughs with 50% ADA, which were similar. This also corresponded to a lower tan δ for Gox relative to the other treatments (Figure 2A). In addition, dough prepared with Glenn was much stronger than the other cultivars as evident by larger |G*| and smaller tan δ values. For Glenn, all doughs appeared stronger than the control when the additive was added. For Harvest, |G*| was slightly higher for all additives at the 100% permitted levels relative to 50% (Figure 4.1B), however similar differences were not seen in the tan δ data (Figure 4.1B).

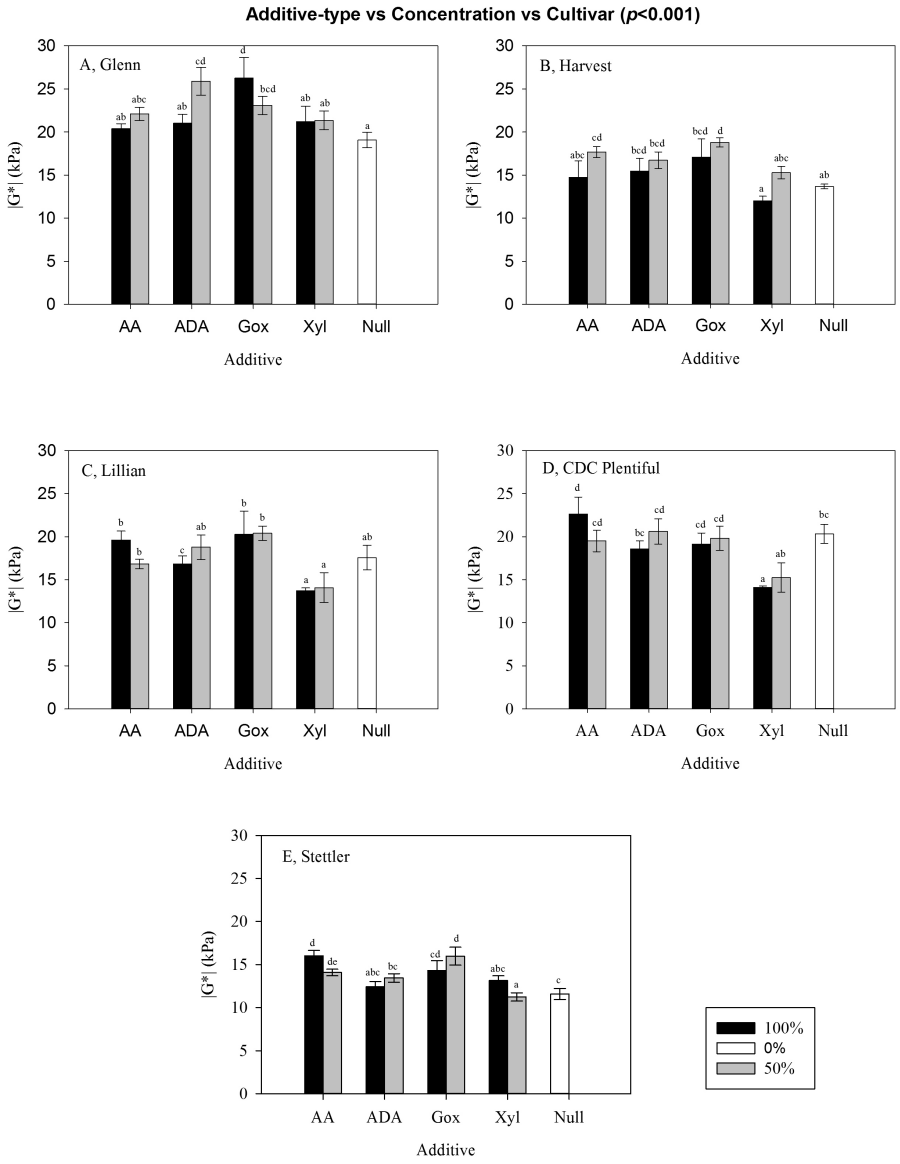

Figure 4.1 The effect of additives, concentration, and wheat cultivar-type on the complex modulus (|G*|) (kPa) (p<0.001). Data represents the mean ± one standard deviation. *Abbreviations*: AA (ascorbic acid); ADA (azodicarbonamide); Gox (glucose oxidase) and Xyl (fungal xylanase). Concentration refers to 50 and 100% of the maximum permitted by Health Canada in foods for chemical additives, and that recommended by a commercial supplier (DuPont) in the case of enzymatic treatments. Different letters represent significant statistical difference from each other ($p \leq 0.05$, Tukey's test).

Doughs with Xyl at the 50% were slightly weaker than that of the control as evident by a lower |G*| and higher tan δ. For Harvest, all doughs were stronger than the control when the additives (oxidizers or enzymes) were added, with the exception of Xyl. In the case of Lillian, dough prepared with AA and ADA showed an additive concentration effect, whereas all other treatments did not (Figure 4.1C). Dough with AA was stronger at the 50% permitted level relative to the 100% level, as evidenced by higher |G*| values, whereas the opposite was true

with ADA. However, both of these doughs (with AA and ADA) did not show any benefit over the control. Doughs prepared with Gox were stronger than the rest, whereas those prepared using Xyl were weaker, including those of the control. Similar trends were observed in the tan δ data (Figure 4.1C). For CDC Plentiful, doughs prepared with AA at the 50% permitted level had higher |G*| then when at the 100% level, and the former was slightly stronger than the control (Figure 4.1D). It was hypothesized that at above the 50% permitted level of AA a saturation level was reached, where above which AA had a negative effect on dough strength. ADA, Gox and Xyl, all had slightly higher |G*| values at the 100% permitted level than the 50% level, however, ADA and Gox were not stronger than the control. The addition of Xyl made the doughs weaker (Figure 4.1D).

Similar trends were observed in the tan δ data (Figure 4.2). Stettler was the weakest of all cultivars with the lowest |G*| and highest tan δ (Figures 4.1 and 4.2). Almost all additives increased the strengths of the dough, with the exception of Xyl. Doughs prepared with AA at the 50% level had higher |G*| and lower tan δ than the 100% permitted level, whereas the opposite trend was seen with ADA and Gox. Correlations between rheology and composition parameters were strong for |G*| in relation to WG and DG (r = -0.85 and -0.90, respectively, $p<0.001$). WG also had a strong correlation with tan δ (r = 0.74, $p<0.001$). Solvent retention capacity (SRC) parameters such as ddH_2O, sodium carbonate and lactic acid had a good correlation with |G*| with r = 0.74, 0.77 and 0.87 ($p<0.001$), respectively. Lactic acid also correlated well with J_{max} (r = 0.77, $p<0.001$).

Differences in creep recovery data were not evident, where the J_{max} and J_{el} mean values were 0.49 ± 0.28 mPa^{-1} and 0.73 ± 0.04, respectively, regardless of the cultivar, additive-type and additive concentration. Although there were no statistical differences, data varied differently with cultivar. For instance, J_{max} and J_{el} value ranged between 0.19-0.34 mPa^{-1} and 0.72-0.77 for Glenn, 0.30-0.94 mPa^{-1} and 0.57-0.77 for Harvest, 0.29-0.81 mPa^{-1} and 0.71-0.78 for Lillian, 0.28-0.68 mPa^{-1} and 0.72-0.76 for CDC Plentiful, and 0.42-1.78 mPa^{-1} and 0.56-0.78 mPa^{-1} for Stettler, respectively (Table 4.3). Principle component analysis of the rheological data found that Harvest and Lillian, Plentiful and Stettler, and Glenn were distinctly different from one another (Figure A4.5, Appendix).

In summary, although |G*| and tan δ showed inversely related trends, the |G*| data appeared to be more sensitive to changes in dough composition. Lower tan δ values suggest a more cross-linked gluten network and better baking quality.

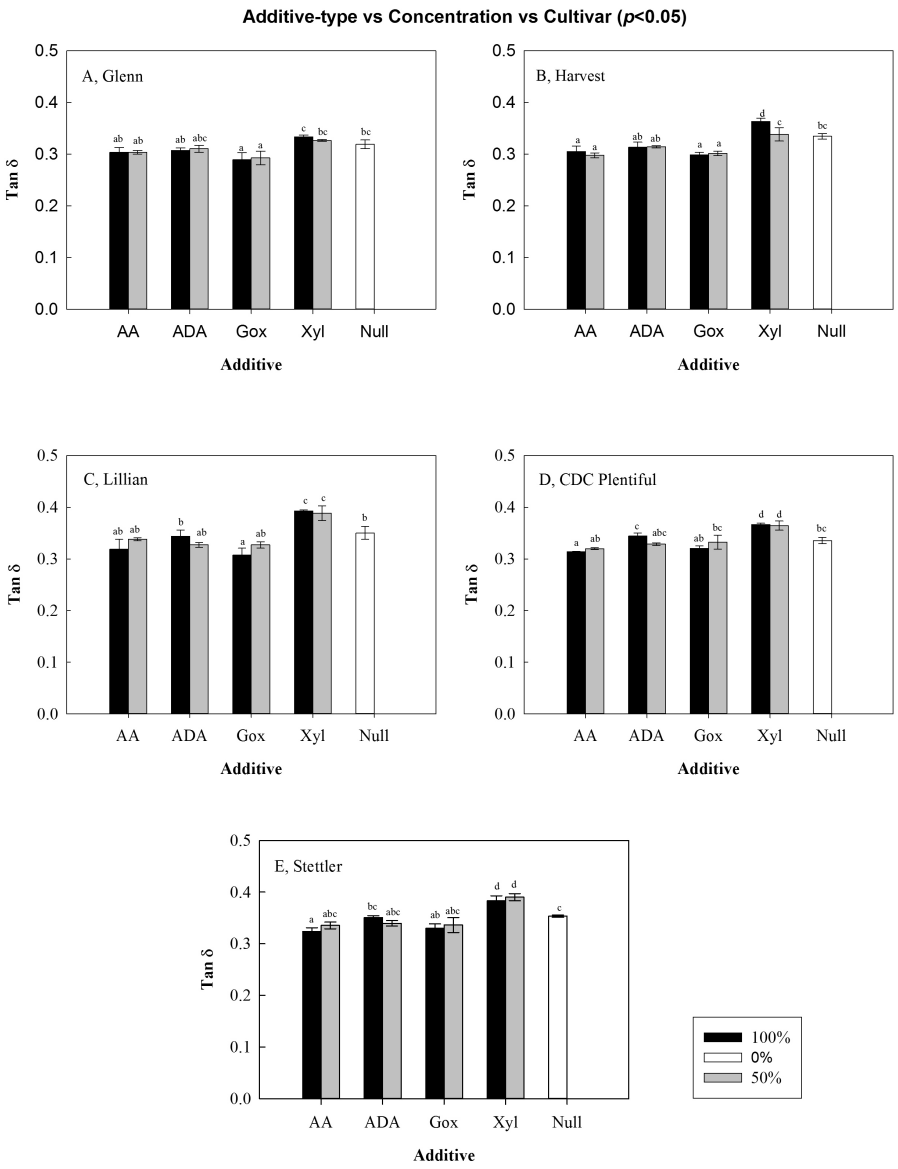

Figure 4.2 The effect of cultivar, concentration, and additive-type interaction on tan δ (p<0.05). Data represents the mean ± one standard deviation. *Abbreviations*: AA (ascorbic acid); ADA (azodicarbonamide); Gox (glucose oxidase) and Xyl (fungal xylanase). Concentration refers to 50 and 100% of the maximum permitted by Health Canada in foods for chemical additives, and that recommended by a commercial supplier (DuPont) in the case of enzymatic treatments. Different letters represent significant statistical difference from each other ($p \leq 0.05$, Tukey's test).

Overall, Gox was most effective at strengthening the dough among the various cultivars as it acts to form a greater amount of protein crosslinks within the gluten network. Gox also gave comparable values as the chemical oxidizers but was more consistent across the different cultivars. Gox catalyzes the oxidation of β-D-glucose in the presence of oxygen to D-gluconic acid and hydrogen peroxide (Joye et al., 2009). Then the hydrogen peroxide oxidizes free sulfhydryl groups to form disulfide bridges and, crosslinks with dityrosine (Joye et al., 2009).

Gox is known to increase strength up to a certain point dependent on the original flour quality, and then over oxidation can lead to a poorer gluten network with less gas retention (Joye et al., 2009; Bonet et al., 2006). Both ADA and AA act to strengthen the gluten network by oxidizing free sulfhydryl (SH) groups to form more disulfide linkages, however they too can over oxidize above an optimum concentration.

In contrast, Xyl had no or an adverse effect on dough strength. Xyl acts to hydrolyze pentosans to increase free water absorption, which indirectly strengthens the network by inducing the re-aggregation of gluten proteins. However, under the present conditions this led to a weaker network. This may be caused by overdosing of the enzyme with the addition of the Xyl prior to mixing, where excessive hydrolysis can result in the redistribution of water from the pentosans to the gluten and starches, giving a weaker gluten network (Butt et al., 2008). In terms of J_{max} and J_{el}, Xyl consistently gave higher J_{max} and lower J_{el} values than Gox, indicating both the dough weakening and strengthening effect of Xyl and Gox, respectively. Effects of additives were cultivar specific, which relates back to their composition. For instance, based on correlations cultivars with higher amounts of damaged starch and gluten proteins led to dough with greater strength. Stronger cultivars were less sensitive to the addition of additives than weaker ones. In some instances, a saturation point was possibly reached with concentration, where above that the presence of the chemical additive or enzyme had a negative effect on dough strength, suggesting the need for optimization of the additive-cultivar relationship in relation to additive-concentration. Differences between AA and ADA reflect the speed in which they react, where the latter is faster than the former (Joye et al., 2009).

4.5. Conclusion

Cultivar-type had a strong effect on dough strength properties (mixograph, micro-doughLAB and rheology). Overall Glenn appeared as the strongest cultivar; as it had a higher mixing time, stability, protein content, and energy required to peak compared to the other cultivars assessed. In contrast, based on the composition, mixograph and microDoughLab parameters, Harvest and Lillian showed weaker dough strength than the other examined cultivars requiring the lowest energy to get to the peak dough development, and highest MTI values, and are possibly less suited to breadmaking that the others in the present study. The fundamental rheology suggests that the use of Gox for improving dough strength is promising, when compared to the control and/or chemical oxidizers. On the other hand, commercial xylanase showed equal or poorer dough strength when compared to the control possibly because of over dosing. Even though the cultivar-type was observed to have a fundamental role

in the results in relation to the oxidizers and enzymes used, more research should be performed to determine the optimal concentration for each cultivar to assess the cost efficiency of its use in substitution of oxidizers, as the impact of each additive is cultivar-specific.

4.6. Linkage

Results from this study suggested higher dependency on wheat cultivar type than additive concentration or type. Therefore, stronger cultivars had naturally higher strength and better dough handling properties. Thus, the addition of enzymes or chemical oxidizers, were effective in improving dough properties when compared to the controls. However, for the CWRS variety registration tests not only cultivar's quality parameters and composition are reported and tested to determine if they can be or not classified in this class. Therefore, a baking performance test is indispensable to further evaluate and understand how the different cultivars were going to behave during the baking process and final products towards the use of additives.

5. EFFECT OF CHEMICAL OXIDIZERS AND ENZYMATIC TREATMENTS ON THE BAKING QUALITY OF DOUGHS FORMULATED WITH FIVE CANADIAN SPRING WHEAT CULTIVARS[2]

5.1. Abstract

For many years, the baking industry has been using chemical improvers as a way for compensating for flour quality variation due to growing conditions or wheat cultivar. However, the replacement of chemical dough improvers with natural ingredients or processing aids (i.e., enzymes) allows for the production of "cleaner label" products. In the present research, dough and bread properties (mixing time, oven rise, loaf volume, crumb firmness and C-cell parameters) were analyzed as a function of wheat cultivar (Glenn, Harvest, Lillian, CDC Plentiful, and Stettler), additive-type (ascorbic acid, azodicarbonamide, glucose oxidase and fungal xylanase) and concentration. Overall, the cultivar Glenn appeared to have improved baking performance relative to the other cultivars, regardless of the additive and additive-concentration. On the other hand, Stettler showed poorer baking quality and performance even with the addition of oxidizers and enzymes in relation to the control. The concentration of additive was found to have little or no effect on improving baking properties within each cultivar. Enzymes had similar or better performance than oxidizers in most cases.

Significance and novelty: Evaluation of the baking performance of different commercialy used enzymes, glucose oxidase and fungal xylanase. Opportunity to have a better understanding of the effects of cultivar type and concentration, as the cultivars represent a range of gluten strength. Thus, provide a better understanding of enzymes as an alternative for the baking industry to create clean labels and more consumer friendly products.

[2] Tozatti, P., Hopkins, E. J., Briggs, C., Hucl, P. Nickerson, M.T. (in press). Effect of chemical oxidizers and enzymatic treatments on the baking quality of doughs formulated with five Canadian spring wheat cultivars. Food Science and Technology International.

5.2. Introduction

Bread is one of the oldest and most widely consumed foods around the globe, contributing substantially to the daily intake of carbohydrates, dietary fiber, minerals, and B-vitamins (Joye et al., 2009). Bread dough is a complex matrix of a variety of ingredients and phases (gases, solids, and liquids). The essential ingredients in bread include wheat flour, salt (NaCl – sodium chloride), water and yeast (e.g., *Saccharomyces cerevisiae*). Whereas, non-essential ingredients include sugar (role: energy, color, and flavor), enzymes (role: bread quality and dough strengthening), dairy products (role: enhances nutrition and color), shortening or fat (role: acts as a softener and dough plasticizer), emulsifying agents (role: plasticizer, softener and bubble stabilizer) and improvers (role: shelf-life and bread quality) (Mucahit, 2012). In order to obtain a good quality bread, a well formed gluten network is needed, capable of retaining carbon dioxide produced by the yeast (Joye et al., 2009). Therefore, bread quality can be determined by the interactions between the ingredients in the process, in addition to their quality and quantity in the formulation. Furthermore, for good quality bread, the dough requires a combination of strength, extensibility and tolerance, that depends mostly on flour quality, water absorption and mixing conditions (Joye et al., 2009).

Deficiencies in wheat quality can be overcome by incorporating exogenous components which alter the functionality of the gluten proteins during the bread making process (Sahi, 2014). Oxidizing agents are widely used in the baking industry for their ability to modify dough properties, such as ascorbic acid, azodicarbonamide, potassium iodate and potassium bromate. Recently, enzymes which favor protein crosslinking (e.g., transglutaminase, glucose oxidase, hexose oxidase and laccase) have been used (Joye et al., 2009). Oxidizing agents tend to minimise SH/SS interchange reactions and, thus, promote the formation of SS bonds within the gluten to alter the strength of the gluten network and its resulting viscoelasticity. Azodicarbonamide (ADA) is one of the fastest oxidants (i.e., reacting minutes after the flour and water are mixed during dough processing) used as a dough improver and as a bleaching agent, acting to strengthen the dough by increasing its resistance to extension (Joye et al., 2009). The mixing time is shortened and less energy input is required to mix the dough. As result, a cohesive dry dough is formed that can tolerate high water absorption (Wieser, 2003). During baking, ADA forms biurea, semicarbazide (SEM) and urazole. ADA may cause allergic reactions (Ye et al., 2011), and the formation of SEM has been linked to the development of some forms of cancer (Kornbrust et al., 2012) and can induce DNA damage (Hirakawa et al., 2003). In addition, other studies reported that oral administration of semicarbazide results in angiomas, angiosarcomas, and lung cancer in mice (Toth, 2000). Currently ADA is under

review in Canada and the United States, while it is banned in the European Union, Australia and New Zealand, and Singapore (EFSA, 2005; Landau, 2014). Commonly known as vitamin C, ascorbic acid is also used as a flour improver in the bread industry (Joye et al., 2009). Ascorbic acid itself is a reducing agent, however, in presence of oxygen and ascorbic acid oxidase, it is converted into the dehydro form, which then takes part in the SH/SS interchange oxidation reaction. As a result, its use typically leads to increased loaf volume and thinner crumb structure (Sahi, 2014).

The ever-increasing demand for more natural products by consumers becoming more self-aware of food products, having less listed chemical additives on ingredient labels, became of great interest in the baking market. Therefore, replacing chemical dough improvers with natural ingredients or processing aids are of interest. For that reason, enzymes appear to be attractive to create "cleaner label" flours as they function in a similar manner and are considered a processing aid enabling companies to keep them off the label (Caballero et al., 2007). Enzymes are a class of proteins that catalyze biochemical reactions by lowering the activation energy resulting in increased reaction rates and no change in reaction equilibrium (Sahi, 2014; Kornbrust et al., 2012). The individual and combined use of a wide range of enzymes (e.g., transglutaminase, glucose oxidase, laccase, α-amylase, pentosanase and protease) in baking is increasing as bakeries attempt to optimize flour functionality, stabilize processing parameters and improve dough quality (Kornbrust et al., 2012; Caballero et al., 2007). The use of enzymes can extend shelf life; improve dough fermentation, dough machinability and stability; increased loaf volume; develop finer and whiter crumb structure; and intensified crust color (Goesaert et al., 2006; Caballero et al., 2007).

Glucose oxidase (Gox) is an oxidizing enzyme, which releases hydrogen peroxide from its catalytic reaction. The Gox treatment modifies gluten proteins by the formation of disulphide and non-disulfide crosslinks. The crosslinks are induced by coupling two cysteine residues within a food protein matrix, resulting in improved viscoelastic and structural properties, as well as better bread making performance (Stoica, 2013; Bonet et al. 2006; Bonet et al., 2007). Gox is an alternative solution to the use of chemical oxidant agents in bread making (Stoica, 2013). Arabinoxylan (AX) is part of the hemicellulose (non-starch polysaccharide) present in wheat flour that accounts for approximately 2.4% of the total dry weight. During breadmaking, almost a third of the water-binding capacity is from AX, therefore it is an important bread quality determinant (McPhillips et al., 2014).

The overall goal of this study was to examine the effect of various strengthening treatments on baking performance of doughs prepared using five commercially grown

Canadian wheat cultivars (Glenn, Harvest, Lillian, CDC Plentiful, and Stettler) representing a range of gluten strengths (weak, intermediate and strong). Commercial enzymes [glucose oxidase (Gox) and xylanase (Xyl)] were used to evaluate their performance compared to chemical oxidizers, such as azodicarbonamide (ADA) and ascorbic acid (AA), which are usually used to strengthen weaker cultivars.

5.3. Materials and methods

5.3.1. Materials

Five Canadian spring wheat cultivars were selected based on their gluten strength. They were classified as weak (Harvest), intermediate (Lillian, CDC Plentiful and Stettler) and strong (Glenn). Each cultivar was grown in uniform unreplicated seed multiplication nursery in the 2017 at the Kernen Crop Research Farm University of Saskatchewan (Saskatoon, SK, Canada). The ascorbic acid and azodicarbonamide were purchased from Sigma-Aldrich Co. (Oakville, ON, Canada); and the enzymes, glucose oxidase (Grindamyl S 758) and fungal xylanase (Grindamyl S250) were donated by DuPont (DuPont Nutrition and Health: New Century, KS, USA).

5.3.2. Flour preparation

Before the milling process, the seed moisture was determined following the AACCI Approved Method 44-15.02. Approximately 5 grams of seeds from each cultivar were milled using a Thomas-Wiley laboratory grinder (model 4, Arthur H. Thomas Co.: Philadelphia, PA, USA) into meal. Based on the determined seed moisture, all wheat cultivars were tempered to 14.5% moisture for ~18 h, and then milled into flour on a Barbender Quadrumat Senior Experimental Mill (Brabender: South Hackensack, NJ, USA). Milling was performed at the University of Saskatchewan in the Grains Innovation Laboratory. Data are presented as the mean ± one standard deviation (n = 3). For baking, the water absorption (FAB) in the flours was determine by the AACCI Approved method 54–21, using a farinograph (C.W. Brabender Instruments, Inc., South Hackensack, NJ, USA) to generate the curves. It used a 50 g mixing bowl in conjunction with the standard operating speed of 63 rpm. The curves were read manually for farinograph water absorption (FAB, 14.0% m.b.).

5.3.3. Breadmaking

For this study the Canadian Short Process (CSP) bake method (a short fermentation method) (Preston et al., 1982) was used. The formulation contained 100 g of wheat flour (14.0%

moisture basis, m.b.), 2.0 g salt, 3.0 g of shortening (Crisco all-vegetable shortening), 4.0 g sugar, 3.0 g fresh compressed yeast (Fleishman compressed yeast), and optimum water (based on farinograph analysis, FAB). FAB values were 61.7% (Harvest), 63.2% (Lillian), 61.4% (Glenn), 59.5% (Stettler) and 60.6% (Plentiful). Chemical oxidizers (AA and ADA), at levels of 50% and 100% of the allowable inclusion levels as specified from Health Canada (2012). Those for the chemical oxidizers are 200 ppm (AA) and 45 ppm (ADA) (Health Canada). For the enzymes, the levels are 150 ppm (Gox) and 300 ppm (DuPont, New Century, KS, U.S.A.). The enzymes (Gox and Xyl) were added at levels of 50% and 100% of the levels recommended by DuPont for the enzymes. All the additives were kept in solution to assure even distribution within the dough.

Ingredients were mixed to slightly past peak in a Swanson Mixer (National Manufacturing Co., Lincoln, NE, USA) at 165 rpm, and peak mixing time (min) and the mixing energy (Wh/kg) were recorded. After mixing, the dough was rounded by hand and placed in a fermentation cabinet (National Manufacturing Co., Lincoln, NE, U.S.A.) which controlled temperature at 34°C and 85% relative humidity. Then, the dough was punched by hand at 15 min, allowed to proof for a further 15 min and then panned at 30 min. The proofing time was determined using baking controls until pre-determined proof height (9 cm). This time (usually ~60 min) was used for all the treatments. After proofing, the loaves were baked for 22 min at 205 ^{o}C (Reel Type Oven, National Manufacturing Co., Lincoln, NE, USA). For each treatment two loaves were baked, with a third repetition done if necessary. Since baking occurred over multiple days/weeks, baking controls were prepared on each day. Using loaf volume as an indicator, a one-way ANOVA showed that there was no significant difference in all controls over the duration of the study ($p>0.05$).

5.3.4. Baking parameters

Right after baking, the loaf height was measured to determine the oven rise (difference in loaf height before and after baking). Then the loaves were cooled at room temperature (21-23^{o}C) for 45 min. Loaf volume (LV) was measured using the rapeseed displacement method with a National Loaf Volumeter (National Manufacturing Company, Lincoln, NB, USA) determined according to the AACCI approved method 10-05.01 and Cathcart and Cole (1938). Each bread loaf was left to cool at room temperature and placed into plastic bags for further crumb structure analysis (~24 h).

Bread crumb firmness was measured following the approved method AACCI 74-09.01. Each bread loaf was sliced transversely into 5 slices to a thickness of 25 mm using a serrated

electric knife (Hamilton Beach 74275RC). Crumb firmness was determined on individual slices of bread using a TA.XT plus Texture Analyzer (Stable Microsystems, Surrey, UK), where a flat ended cylindrical probe of 36 mm in diameter was pushed into the bread at a speed of 2 $mm.s^{-1}$ to a total distance of 8 mm (a local engineering strain of 53%), and the force was recorded (gram force, gF). Data was presented as mean and standard deviation from three slices of each of two separate loaves.

Crumb structure characteristics of bread was analyzed using a C-cell monochrome (CC.300) imaging system (Calibre Control International, UK) according to the approved method by AACC International method 10-18.01. The equipment was calibrated using a calibration card (CC001) for a monochrome system. The monochrome system takes a side-lit image in 256 grayscale and applies software algorithms to determine the internal crumb structure parameters. Each loaf of bread was cut into five slices of one inch (~2.5 cm) using an electric knife (Hamilton Beach 74275RC) ~24 h after it was placed in the plastic bag at room temperature (21–23 ^{o}C), where the middle slice was again cut in 1.25 cm to be analyzed in the C-cell equipment. The sample was placed in the center of the sample-holding tray with the surface for analysis facing upward. The C-Cell monochrome system generates 48 numerical results, of which only seven (7) were focused on for this study, such slice area (mm^2), slice brightness, cell contrast, number of cells, area of cells (%), cell wall thickness (mm), and cell diameter (mm). Data was presented as mean and standard deviation from two separate loaves for each cultivar, additive type and different concentrations (50 and 100%).

5.3.5. Statistics

A 3-way analysis of variance (ANOVA) analysis was performed to test the significance of the main effects (i.e., cultivar, additive-type and or additive-concentration), 2-way interactions (i.e., cultivar x additive-type, cultivar x additive-concentration, and additive-type x additive-concentration), and the 3-way interaction (i.e., cultivar x additive-type x additive-concentration). In addition, one-way ANOVA and a Tukey Post-Hoc test were performed within each cultivar to determine differences between additive and concentration. Principal component analysis (PCA) was performed on the baking parameters (i.e., mixing time, loaf volume, oven rise and crumb firmness). All statistical analysis was performed using SPSS Grad Pack v24 software. A one-way ANOVA was also performed on loaf volume data collected for the baking controls over multiple days/weeks to prove that there was no ‘day effect’ occurring during the baking studies.

5.4. Results and discussion

5.4.1. Mixing time

Mixing time for all dough systems is reported in Table 5.1. In the case of mixing time, only effects of cultivar (p<0.001) and additive-type (p<0.05) were found to be significant (Table A5.1, Appendix). Figure 5.1 depicts the effects associated with the significant main effects. In the case of cultivars, the mixing time was found to be greatest for dough prepared using Glenn flour regardless of the additive or concentration (Figure 5.1A), followed by Stettler, CDC Plentiful, Lillian and Harvest. Generally, higher mixing time is related to higher dough strength, conferred by a stronger gluten network.

Table 5.1. Baking properties of five wheat cultivars and additive/concentration. Data represents the mean values from duplicate bakes ± one standard deviation (n = 2).

Cultivar	Additive	Conc.	Mixing time (min)	Oven rise (cm)	Loaf volume (cm^3)	Crumb firmness (gF)
Glenn	Null	-	6.1 ± 0.3^{a}	3.3 ± 0.4^{ab}	1148 ± 4^{b}	115 ± 21^{a}
	AA	50%	6.7 ± 0.0^{a}	3.3 ± 0.0^{ab}	1105 ± 14^{b}	127 ± 2^{a}
		100%	6.1 ± 0.3^{a}	3.8 ± 0.6^{b}	1215 ± 14^{c}	100 ± 24^{a}
	ADA	50%	6.4 ± 0.5^{a}	3.0 ± 0.4^{ab}	1125 ± 35^{b}	102 ± 1^{a}
		100%	6.1 ± 0.1^{a}	1.8 ± 0.4^{a}	928 ± 11^{a}	138 ± 20^{a}
	Gox	50%	7.4 ± 1.6^{a}	2.8 ± 0.4^{ab}	1135 ± 28^{b}	107 ± 7^{a}
		100%	6.6 ± 0.1^{a}	2.6 ± 0.0^{ab}	1090 ± 0^{b}	110 ± 1^{a}
	Xyl	50%	7.3 ± 0.9^{a}	3.2 ± 0.6^{ab}	1225 ± 0^{c}	102 ± 3^{a}
		100%	7.6 ± 2.1^{a}	3.3 ± 0.4^{ab}	1223 ± 4^{c}	89 ± 5^{a}
	Average		**6.7**	**3.0**	**1133**	**110**
	Min		**6.1**	**1.8**	**928**	**89**
	Max		**7.6**	**3.8**	**1225**	**138**
Harvest	Null	-	3.8 ± 0.2^{a}	2.3 ± 0.1^{cde}	1022 ± 25^{abc}	139 ± 11^{a}
	AA	50%	3.9 ± 0.0^{a}	2.6 ± 0.3^{de}	1030 ± 14^{abc}	141 ± 2^{a}
		100%	3.8 ± 0.2^{a}	2.8 ± 0.1^{e}	1100 ± 0^{c}	133 ± 15^{a}
	ADA	50%	3.8 ± 0.1^{a}	2.3 ± 0.0^{bcd}	1040 ± 7^{abc}	137 ± 7^{a}
		100%	3.8 ± 0.0^{a}	1.8 ± 0.1^{ab}	970 ± 14^{a}	156 ± 5^{a}
	Gox	50%	3.9 ± 0.1^{a}	2.0 ± 0.0^{a}	1015 ± 21^{abc}	144 ± 2^{a}
		100%	3.8 ± 0.4^{a}	1.8 ± 0.0^{abc}	1025 ± 35^{ab}	134 ± 2^{a}
	Xyl	50%	4.0 ± 0.1^{a}	2.5 ± 0.1^{abcd}	1057 ± 32^{bc}	130 ± 18^{a}
		100%	3.9 ± 0.1^{a}	2.3 ± 0.1^{bcd}	1008 ± 3^{ab}	142 ± 15^{a}
	Average		**3.9**	**2.3**	**1030**	**139**
	Min		**3.8**	**1.8**	**970**	**130**
	Max		**4.0**	**2.8**	**1100**	**142**

Table 5.1. Cont.…

Cultivar	Additive	Conc.	Mixing time (min)	Oven rise (cm)	Loaf volume (cm^3)	Crumb firmness (gF)
Lillian	**Null**	**-**	**4.1 ± 0.1^{a}**	**2.4 ± 0.2^{abc}**	**1052 ± 3^{abc}**	**127 ± 10^{b}**
	AA	50%	3.8 ± 0.2^{a}	3.4 ± 0.1^{cd}	1148 ± 4^{bcd}	110 ± 2^{ab}
		100%	3.8 ± 0.4^{a}	3.5 ± 0.6^{d}	1128 ± 3^{d}	118 ± 8^{ab}
	ADA	50%	3.8 ± 0.4^{a}	2.6 ± 0.3^{abcd}	1100 ± 14^{c}	114 ± 2^{ab}
		100%	3.8 ± 0.1^{a}	2.0 ± 0.1^{ab}	1020 ± 14^{ab}	120 ± 10^{b}
	Gox	50%	3.7 ± 0.0^{a}	2.3 ± 0.1^{abcd}	1115 ± 50^{cd}	112 ± 5^{ab}
		100%	4.2 ± 0.2^{a}	1.9 ± 0.3^{abc}	1000 ± 0^{a}	135 ± 15^{bc}
	Xyl	50%	4.1 ± 0.4^{a}	3.0 ± 0.0^{bcd}	1180 ± 28^{d}	88 ± 4^{a}
		100%	3.8 ± 0.2^{a}	3.1 ± 0.1^{bcd}	1148 ± 4^{d}	110 ± 4^{ab}
	Average		**3.9**	**2.7**	**1099**	**115**
	Min		**3.7**	**1.9**	**1000**	**88**
	Max		**4.1**	**3.1**	**1180**	**135**
CDC Plentiful	**Null**	-	4.2 ± 0.1^{a}	2.6 ± 0.0^{a}	1053 ± 4^{a}	158 ± 15^{a}
	AA	50%	4.4 ± 0.0^{a}	3.5 ± 0.1^{a}	1203 ± 4^{c}	139 ± 9^{a}
		100%	4.3 ± 0.2^{a}	3.4 ± 0.1^{a}	1198 ± 18^{c}	157 ± 6^{a}
	ADA	50%	4.2 ± 0.4^{a}	2.3 ± 0.1^{a}	1050 ± 0^{a}	136 ± 18^{a}
		100%	3.8 ± 0.3^{a}	2.7 ± 0.6^{a}	1075 ± 36^{ab}	143 ± 4^{a}
	Gox	50%	4.4 ± 0.1^{a}	3.3 ± 0.6^{a}	1145 ± 21^{abc}	128 ± 25^{a}
		100%	4.5 ± 0.1^{a}	2.8 ± 0.1^{a}	1128 ± 68^{abc}	129 ± 10^{a}
	Xyl	50%	4.1 ± 0.0^{a}	2.8 ± 0.4^{a}	1175 ± 0.0^{bc}	111 ± 6^{a}
		100%	4.2 ± 0.2^{a}	2.9 ± 0.8^{a}	1160 ± 21^{abc}	112 ± 9^{a}
	Average		**4.2**	**2.9**	**1132**	**135**
	Min		**3.8**	**2.3**	**1050**	**111**
	Max		**4.5**	**3.4**	**1203**	**158**
Stettler	**Null**	-	4.3 ± 0.0^{a}	1.9 ± 0.1^{a}	960 ± 21^{a}	180 ± 12^{b}
	AA	50%	5.1 ± 0.2^{c}	2.3 ± 0.1^{ab}	958 ± 25^{a}	171 ± 10^{ab}
		100%	4.6 ± 0.0^{abc}	2.7 ± 0.4^{b}	1063 ± 11^{a}	145 ± 19^{ab}
	ADA	50%	4.5 ± 0.1^{abc}	2.3 ± 0.1^{ab}	1028 ± 4^{a}	152 ± 6^{ab}
		100%	4.4 ± 0.1^{ab}	2.3 ± 0.1^{ab}	1010 ± 14^{a}	156 ± 7^{ab}
	Gox	50%	4.8 ± 0.2^{abc}	1.7 ± 0.1^{a}	1010 ± 92^{a}	146 ± 7^{ab}
		100%	4.5 ± 0.1^{abc}	1.7 ± 0.1^{a}	965 ± 14^{a}	159 ± 8^{ab}
	Xyl	50%	5.0 ± 0.3^{cd}	2.3 ± 0.1^{ab}	1060 ± 7^{a}	129 ± 14^{a}
		100%	4.7 ± 0.3^{bc}	2.3 ± 0.1^{ab}	990 ± 28^{a}	142 ± 15^{ab}
	Average		**4.7**	**2.2**	**1005**	**153**
	Min		**4.3**	**1.7**	**958**	**142**
	Max		**5.0**	**2.7**	**1063**	**180**

Abbreviations: AA (ascorbic acid); L-Cys (L-cysteine); Gox (glucose oxidase); ADA (azodicarbonamide); and Xyl (fungal xylanase)
Data within the same colunm with different letters are significantly different (p<0.05).

Overall, AA and ADA seemed to have little or no effect on mixing time relative to the control. Because ADA is fast acting, it oxidizes sulfhydryl groups upon the addition of water, reducing the mixing time (Wieser, 2003). In contrast, the addition of enzymes (Gox) and (Xyl)

led to a slight dough mixing time increase (p<0.05) (Figure 5.1B). Preston et al. (2001) studied different Canadian Western Red Spring (CWRS) cultivars and showed considerable variation in dough strength based on the Canadian short process (CSP) mixing time, where cultivar and the environment strongly influenced mixing time results.

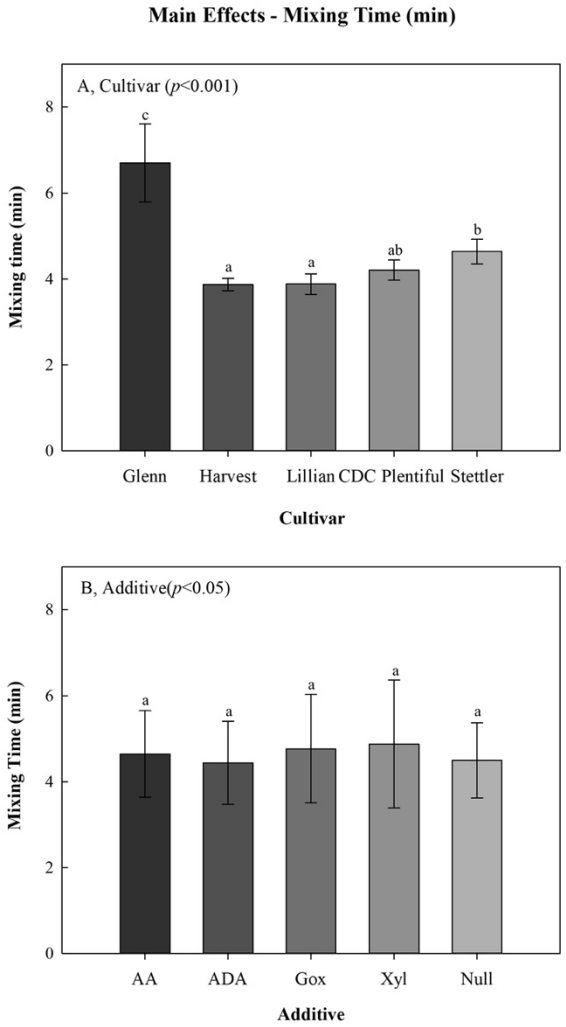

Figure 5.1 The effect of wheat cultivar (p<0.001) (A) and strengthening additive (p<0.05) (B) on the mixing time of resulting doughs. Data represent the mean ± one standard deviation (n = 4 for all additives; n = 2 for the no treatment controls). *Abbreviations*: Null (no treatment), AA (ascorbic acid), ADA (azodicarbonamide), Gox (glucose oxidase), and Xyl (fungal xylanase). Different letters represent significant statistical difference from each other (p ≤ 0.05, Tukey's test).

5.4.2. Oven rise

Oven rise measures the height increase during baking (difference between the height right before and after baking) and is presented in Table 5.1 for all treatments. A 3-way ANOVA was performed for oven rise for all formulations which determined the main effects of cultivar (p<0.001) and additive-type (p<0.001) to be significant, along with the 2-way interactions of cultivar x additive-type (p<0.01) and additive-type x concentration (p<0.05) (Table A5.1,

Appendix). Overall, Glenn was found to have the highest oven rise (3.0 cm), followed by CDC Plentiful (2.9 cm), Lillian (2.7 cm), Harvest (2.3 cm) and then Stettler (2.2 cm), regardless of the additive type or concentration. Considering, the interaction term of cultivar x additive-type (p<0.01), the addition of AA and Xyl resulted similar trends with higher oven rises being seen for Glenn, Lillian and CDC Plentiful, with lower rises in the case of Harvest and Stettler (Figure 5.2A).

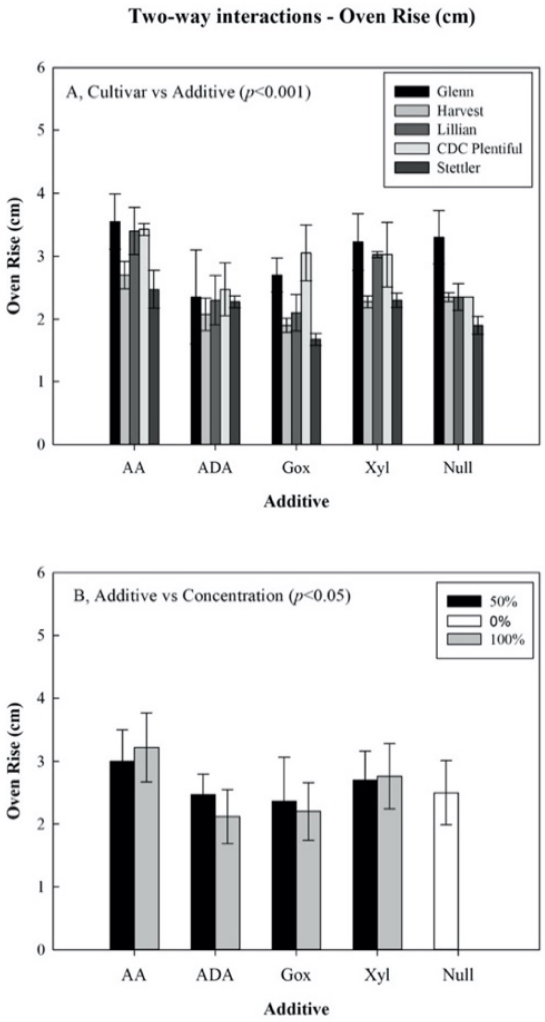

Figure 5.2. The effect of additives and wheat cultivar on the oven rise (cm) of the resulting doughs (p<0.001) (A) (n = 4), and the effect of additive treatment and their concentration on the oven rise (cm) of resulting doughs (p<0.05) (B) (n = 10). Data represent the mean ± one standard deviation. Abbreviations: NT (no treatment); AA (ascorbic acid); ADA (azodicarbonamide); Gox (glucose oxidase and Xyl (fungal xylanase). Concentration refers to 50 and 100% of the maximum permitted by Health Canada in foods for chemical additives, and that recommended by a commercial supplier (DuPont) in the case of enzymatic treatments. Different letters represent significant statistical difference from each other (p ≤ 0.05, Tukey's test).

The oven rise was greater than the control for Lillian and CDC Plentiful, but not for Glenn. In the case of ADA, no difference was seen for oven rise between cultivars, with all

being similar to the control with the exception of Glenn, which was reduced (Figure 5.2A). In the case of Gox, CDC Plentiful was most sensitive giving a higher oven rise than the control, whereas all others experience reduced oven rise in the presence of Gox (Figure 5.2A). One of the products from glucose oxidase reaction is hydrogen peroxide (H_2O_2), which in combination with peroxidase (native to wheat flour) may cause water-soluble pentosans in the dough to gel. This gelation can limit water mobility, resulting in dryer dough, change in dough rheology and, therefore, lower and limited oven rise (Vemulapalli et al., 1998). For Xyl, oven rise was improved in the case of Lillian and CDC Plentiful over the control, which may be due to its increased dough stability, which resulted in prolonged oven rise during the first stage of baking (Goesaert et al., 2005). Other treatments, however, were similar in magnitude to the control (Figure 5.2A).

In terms of the significant interaction between additive-type and concentration ($p<0.05$), oven rise was higher at the 50% permitted levels for ADA and Gox than at the 100% level, whereas the reverse was true for AA and Xyl although differences were minor (Figure 5.2B). Yamada and Preston (1992) also reported that a further increase in ADA and AA concentration did not significantly change the maximum oven rise or loaf volume. In addition, oven rise was only greater than the control when AA was added, with all other additives leading to similar values to the control (Figure 5.2B).

5.4.3. Loaf volume

Loaf volume for all treatments is presented in Table 5.1 and Figure 5.3. From the ANOVA analysis, all main effects were significant ($p<0.001$), along with the 2-way interactions for cultivar x additive-type ($p<0.001$), cultivar-type x concentration ($p<0.05$) and additive x concentration ($p<0.001$), and the 3-way interaction between cultivar x additive-type x concentration ($p<0.001$) (Table A5.1, Appendix). Overall, loaf volume was found to be positively correlated with oven rise ($r = 0.81$, $p<0.01$).

In the case of Glenn, loaf volume was found to increase relative to the control in the presence of Xyl (regardless of the concentration) and AA at the 100% concentration. All other treatments were similar to that of the control with the exception of ADA at the 100% level where a reduction in loaf volume occurred (Figure 5.3A). The lower volume at the 100% level could be related to an over-treatment (optimum concentration is suggested to be 10 to 20 ppm) which results in poor volume and crumb characteristics, resulting from a tight, extensible dough (Wieser, 2003). For Harvest, although there were slight differences in loaf volume between treatments, none were significantly different from the control (Figure 5.3B).

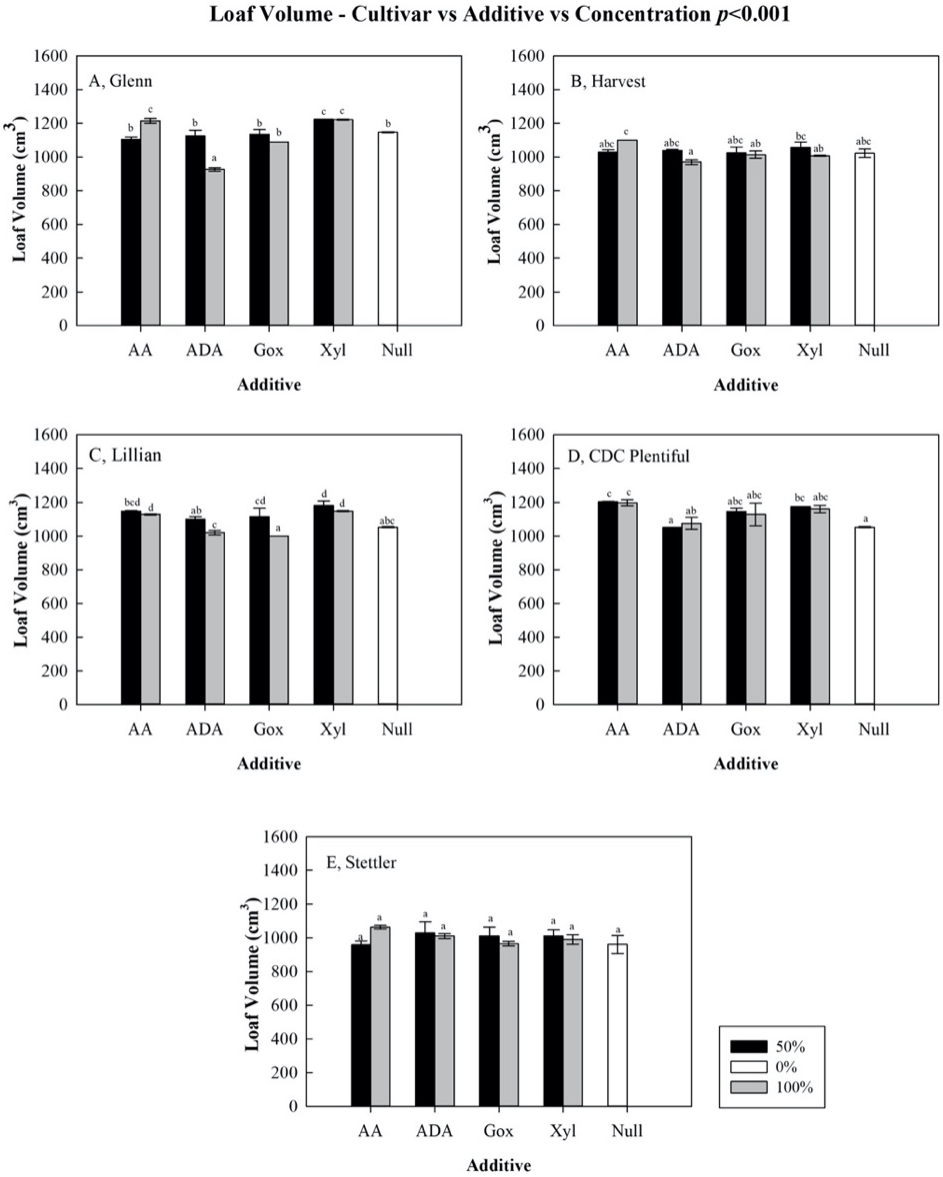

Figure 5.3. The effect of strengthening additives (type and concentration) and wheat cultivar on loaf volume (p<0.001). Data represent the mean ± one standard deviation (n = 2). *Abbreviations*: AA (ascorbic acid); ADA (azodicarbonamide); Gox (glucose oxidase); Xyl (fungal xylanase) and Null (no treatment). Concentration refers to 50 and 100% of the maximum permitted by Health Canada in foods for chemical additives, and that recommended by a commercial supplier (DuPont) in the case of enzymatic treatments. Different letters represent significant statistical difference from each other ($p \leq 0.05$, Tukey's test).

For Lillian, the addition of AA and Xyl (regardless of the level), and Gox at the 50% level resulted in higher loaf volumes compared to the control (Figure 5.3C). In the case of ADA and Gox, a slight reduction in loaf volume was observed as the added levels increased from 50 to 100% (Figure 5.3C). This reduction could be related to an overdosage which can lead to a negative effect on the handling characteristics of dough and the quality of the resulting bread

(Bonet et al., 2006). In addition, when compared to the control, the results were in accordance to Rasiah et al. (2005), who did not observe significant differences between loaf volume in the presence or absence of Gox. For CDC Plentiful, the addition of AA (regardless of the level) and Xyl at the 50% level only were able to increase loaf volume relative to the control (Figure 5.3D), whereas all other treatments were similar to the control. For Stettler, all treatments had similar loaf volumes as the control (Figure 5.3E).

Similar results were found by Yamada and Preston (1992), comparing different oxidizers on CWRS cultivars, where no significant difference in loaf volume in relation to additive was found when the authors used the optimal concentration for each additive (ADA 40 ppm, AA 125 ppm). The same trend was found when the authors used a wide range of AA concentrations (125-200 ppm) and found no significant effect on loaf volume (Yamada and Preston, 1992). The addition of AA and Xyl resulted in a consistent higher loaf volume compared to the control for all the cultivars reaching as high as 1215 and 1225 cm^3 (Glenn).

In most of the cases, the addition of enzymes resulted in increased loaf volume in comparison to the control and/or chemical oxidizers. An increase in loaf volume due to the addition of xylanase was also reported in other studies (Shah et al., 2006; Ahmad et al., 2014), which could be related to the water redistribution from pentosans to the gluten network, increasing the gluten functionality and, consequently, resulting in a more extensible dough and high loaf volume (Ahmad et al., 2014). In the current study, similar or improved loaf volume was found with the addition of Gox in comparison to ADA, AA and the control. These results were not unexpected since previous studies had confirmed the beneficial effect of Gox addition in increasing loaf volume (Kriaa et al., 2016). Except for Lillian, for all other cultivars the different concentration did not have a significant effect on Gox (p>0.05), which contradicts Bonet et al. (2006) who stated that the effect of Gox depends on the concentration. Gox poorer loaf volume could be also related to the short-time baking process, where the level of Gox required to obtain optimum strengthening is too high, leading into an overoxidized and dryer dough, due to the oxidative gelation of water-soluble pentosans which limits the water mobility within the dough (Vemulapalli et al., 1998).

Overall, the effect of the additives was dependent upon the wheat cultivar type, due to their variation in protein content and composition, damaged starch, and other quality parameters which directly affect dough properties and baking performance. Despite Harvest being considered the weakest cultivar, the volume of loaves prepared with Harvest were similar to those of Lillian and CDC Plentiful, while the loaves prepared with Stettler flour had the

lowest loaf volume and were not affected by additive addition. In particular, the stronger cultivar (Glenn), showed less variation in loaf volume than the other wheat cultivars.

5.4.4. Crumb firmness

Crumb firmness as a function of strengthening treatments (additive-type and additive-concentration) and wheat cultivar is presented in Table 5.1 and Figure 5.4. The 3-way ANOVA analysis found that all main effects, cultivar (p<0.001), additive-type (p<0.001) and additive-concentration (p<0.05) to be significant. The 2-way interaction between additive x concentration, and the 3-way interactions were also found to be significant (p<0.05) for crumb firmness (Table A5.1, Appendix). Typically, higher crumb firmness values are a result of more strength required to be applied to the bread slice. In the case of Glenn, Harvest and CDC Plentiful no differences in crumb firmness were seen among any of the treatments relative to the control (Figure 4A, B, D). For Lillian, Xyl at the 50% level caused a reduction in crumb firmness, whereas all other treatments were similar to that of the control (Figure 5.4C). In contrast, crumb firmness was reduced at the 50% Xyl level relative to the control, whereas all other treatments were similar. Furthermore, as the addition of xylanase is known to increase dough stability, it allows the dough to maintain an optimal volume for a longer time, as a result a prolonged oven rise during the first stage of baking leads to higher loaf volume and a finer, softer and more homogeneous bread crumb (Goesaert et al., 2005). Jiang et al. (2005) reported a decrease in crumb firmness with the addition of 100 ppm of fungal xylanase, which had the lowest crumb firmness values. Lower crumb firmness results in a softer crumb, which could be explained by increase in loaf volume (Amita et al., 2006).

No additive or concentration had a positive effect on Stettler when compared to the control values, they were lower or significantly similar to the control (p<0.05). Gox addition resulted in similar or lower crumb firmness values compared to the control for all cultivars and both concentrations, which has also been observed in other studies (Kriaa et al., 2016). In most cases, ADA at the 100% level had higher crumb firmness values which were similar or greater than the control, indicating softer structure. The softer crumb could be attributed to the fact that ADA can tolerate high water absorption while resulting in a good texture and volume of the loaf (Goesaert et al., 2005).

Overall, crumb firmness was found to be negatively correlated with loaf volume (r = -0.68, p<0.01) indicating that the bigger the loaf, the softer the crumb. Principle component analysis of all baking parameters suggest loaves prepared from Harvest and Stettler flours are closely related, and different than loaves produced by Plentiful and Lillian, which also were

closely related. Loaves produced by Glenn flour were distinctly different from all flours (Figure A5.1, Appendix).

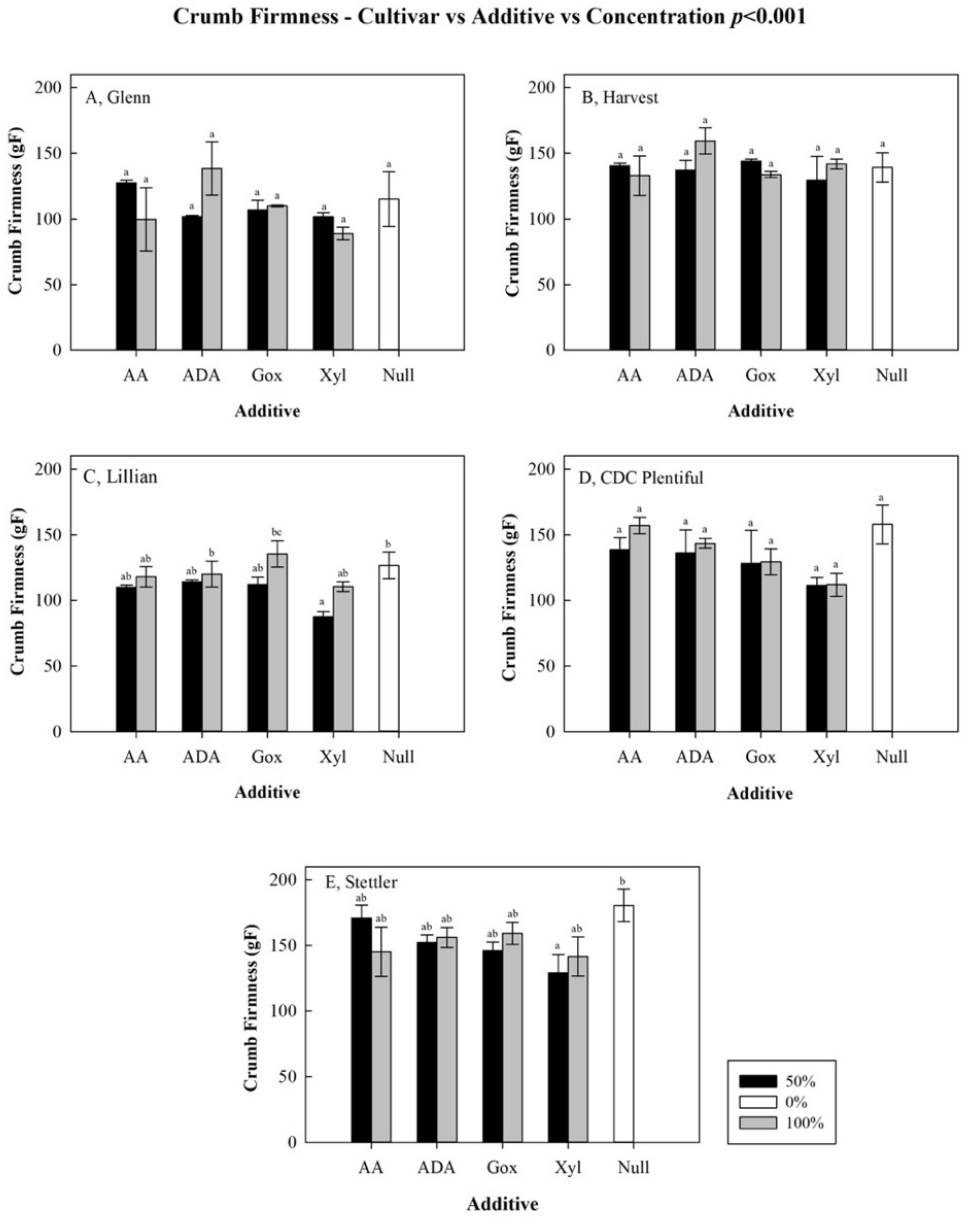

Figure 5.4. The effect of strengthening additives (type and concentration) and wheat cultivar on crumb firmness ($p<0.001$). Data represent the mean ± one standard deviation (n = 2). Abbreviations: AA (ascorbic acid); ADA (azodicarbonamide); Gox (glucose oxidase); Xyl (fungal xylanase) and Null (no treatment). Concentration refers to 50 and 100% of the maximum permitted by Health Canada in foods for chemical additives, and that recommended by a commercial supplier (DuPont) in the case of enzymatic treatments. Different letters represent significant statistical difference from each other ($p \leq 0.05$, Tukey's test).

5.4.5. Relationship between c-cell results and loaf quality

The crumb structure parameters obtained from the C-cell image analyses are summarized in Table 5.2 for all treatments. The C-cell uses image analysis software to evaluate texture, color, and appearance of baked products. Figure A5.2 (Appendix) represents C-cell imaging of bread loaves from the five wheat cultivars containing no additives. A 3-way ANOVA was performed for all C-cell parameters and determined only slice area to have a significant 3-way interaction term ($p<0.001$) (Table A5.2, Appendix). Slice area was also found to be positively correlated with oven rise ($r = 0.81$, $p<0.01$) and loaf volume ($r = 0.93$, $p<0.01$) and, negatively correlated to crumb firmness ($r = -0.63$, $p<0.01$). The high correlation between slice area, oven rise, and loaf volume may be related to the bread dough strain hardening, that allows the gas cell walls to expand and resist rupturing and having a better stability. This results in a finer crumb structure and larger baked volume in comparison to doughs with poorer strain hardening properties (Dobraszczyk and Morgenstern, 2003). Consequently, the crumb structure is affected, where the higher the crumb firmness, more strength needs to be applied and, usually, lower volume as the cells resist expansion – which explains the negative correlation.

An ANOVA for the number of cells indicated significant main effects of cultivar and additive-type ($p<0.001$), and significant ($p<0.05$) 2-way interactions between cultivar x additive and additive-type x concentration ($p<0.05$). Cell number also correlated positively with oven rise ($r = 0.69$, $p<0.01$) and negatively with loaf volume ($r = 0.63$, $p<0.01$) indicating that as the loaf became bigger there was an increasing number of cells. Similar correlation between loaf volume and number of cells was presented by Yovchev et al. (2017), when analyzing 37 different Canadian western red spring (CWRS) cultivars at two different salt levels. Although other parameters such as slice brightness, cell contrast, area of cells, cell wall thickness and cell diameter showed some significant main effects or two-way interactions (Table 5.2), they were considered minor and did not correlate with the baking attributes.

Table 5.2. C-cell imaging of bread from five wheat cultivars and differing additive/concentrations. Data represents the mean from duplicate bakes ± standard deviation (n=2).

Cultivar	Additive	Conc.	Slice area (mm^2)	Slice brightness	Cell contrast	Number of cells	Area of cells (%)	Cell wall thickness (mm)	Cell diameter (mm)
Glenn	Null	-	8130 ± 46^{ab}	146 ± 1^{a}	0.70 ± 0.03^{a}	4419 ± 156^{a}	55.4 ± 0.3^{ab}	0.45 ± 0.01^{a}	2.31 ± 0.01^{ab}
	AA	50%	7971 ± 142^{ab}	145 ± 2^{a}	0.70 ± 0.02^{a}	4491 ± 310^{a}	55.3 ± 0.4^{ab}	0.44 ± 0.02^{a}	2.21 ± 0.07^{ab}
		100%	8443 ± 399^{ab}	145 ± 2^{a}	0.72 ± 0.01^{a}	4840 ± 200^{a}	55.5 ± 0.6^{ab}	0.44 ± 0.00^{a}	2.25 ± 0.05^{ab}
	ADA	50%	7955 ± 243^{ab}	146 ± 2^{a}	0.71 ± 0.01^{a}	4154 ± 184^{a}	55.7 ± 0.5^{ab}	0.46 ± 0.00^{a}	2.46 ± 0.04^{bc}
		100%	6712 ± 37^{a}	139 ± 1^{a}	0.69 ± 0.00^{a}	3451 ± 217^{a}	56.4 ± 0.3^{b}	0.46 ± 0.01^{a}	2.62 ± 0.01^{c}
	Gox	50%	8229 ± 129^{ab}	146 ± 1^{a}	0.73 ± 0.00^{a}	4895 ± 181^{a}	54.9 ± 0.1^{a}	0.43 ± 0.01^{a}	2.16 ± 0.01^{a}
		100%	7811 ± 27^{ab}	145 ± 1^{a}	0.72 ± 0.01^{a}	4556 ± 147^{a}	55.2 ± 0.3^{ab}	0.44 ± 0.01^{a}	2.17 ± 0.10^{a}
	Xyl	50%	8371 ± 441^{ab}	142 ± 2^{a}	0.70 ± 0.01^{a}	4427 ± 585^{a}	55.8 ± 0.3^{ab}	0.45 ± 0.01^{a}	2.37 ± 0.04^{abc}
		100%	8593 ± 21^{ab}	143 ± 4^{a}	0.69 ± 0.02^{a}	4686 ± 554^{a}	56.3 ± 0.3^{b}	0.44 ± 0.02^{a}	2.35 ± 0.13^{ab}
		Average	**8024**	**144**	**0.71**	**4435**	**49.4**	**45**	**2.32**
		Min	**6712**	**139**	**0.69**	**3451**	**55.2**	**0.43**	**2.16**
		Max	**8593**	**146**	**0.73**	**4895**	**56.3**	**0.46**	**2.46**
Harvest	Null	-	7361 ± 161^{b}	148 ± 0^{a}	0.72 ± 0.00^{b}	3997 ± 44^{abc}	55.4 ± 0.4^{a}	0.45 ± 0.01^{a}	2.30 ± 0.15^{abc}
	AA	50%	7701 ± 127^{b}	144 ± 4^{a}	0.71 ± 0.01^{ab}	4234 ± 249^{bc}	55.2 ± 0.9^{a}	0.45 ± 0.01^{a}	2.22 ± 0.04^{ab}
		100%	7900 ± 74^{b}	145 ± 1^{a}	0.73 ± 0.00^{b}	4700 ± 90^{c}	55.4 ± 0.1^{a}	0.43 ± 0.00^{a}	2.12 ± 0.02^{a}
	ADA	50%	7417 ± 7.1^{b}	145 ± 2^{a}	0.71 ± 0.01^{ab}	3368 ± 40^{ab}	56.0 ± 0.6^{a}	0.49 ± 0.00^{a}	2.75 ± 0.12^{bc}
		100%	6708 ± 322^{a}	143 ± 0^{a}	0.68 ± 0.01^{a}	3071 ± 80^{a}	57.0 ± 0.9^{a}	0.48 ± 0.01^{a}	2.89 ± 0.24^{c}
	Gox	50%	7418 ± 129^{b}	146 ± 1^{a}	0.73 ± 0.02^{b}	3477 ± 746^{ab}	55.5 ± 0.7^{a}	0.48 ± 0.04^{a}	2.58 ± 0.23^{abc}
		100%	7511 ± 161^{b}	145 ± 2^{a}	0.71 ± 0.01^{ab}	3838 ± 262^{abc}	55.5 ± 0.2^{a}	0.46 ± 0.02^{a}	2.40 ± 0.22^{abc}
	Xyl	50%	7408 ± 152^{b}	146 ± 2^{a}	0.71 ± 0.01^{ab}	3799 ± 67^{abc}	55.8 ± 0.1^{a}	0.46 ± 0.01^{a}	2.46 ± 0.04^{abc}
		100%	7325 ± 144^{ab}	144 ± 2^{a}	0.70 ± 0.00^{ab}	3952 ± 230^{abc}	56.0 ± 0.3^{a}	0.45 ± 0.01^{a}	2.43 ± 0.11^{abc}
		Average	**7417**	**145**	**0.71**	**3826**	**55.8**	**0.46**	**2.46**
		Min	**6708**	**143**	**0.68**	**3071**	**55.2**	**0.43**	**2.22**
		Max	**7900**	**148**	**0.73**	**4700**	**57.0**	**0.49**	**2.89**

Table 5.2. Cont..

Cultivar	Additive	Conc.	Slice area (mm^2)	Slice brightness	Cell contrast	Number of cells	Area of cells (%)	Cell wall thickness (mm)	Cell diameter (mm)
Lillian	Null	-	7625 ± 9^{abc}	149 ± 1^{a}	0.70 ± 0.03^{a}	4110 ± 372^{a}	55.5 ± 0.1^{ab}	0.45 ± 0.02^{a}	2.39 ± 0.14^{a}
	AA	50%	8218 ± 39^{cd}	153 ± 2^{a}	0.71 ± 0.02^{a}	4765 ± 339^{a}	55.1 ± 0.3^{ab}	0.44 ± 0.01^{a}	2.19 ± 0.11^{a}
		100%	8124 ± 158^{bcd}	149 ± 4^{a}	0.71 ± 0.02^{a}	5041 ± 428^{a}	54.7 ± 0.5^{ab}	0.43 ± 0.01^{a}	2.07 ± 0.14^{a}
	ADA	50%	7871 ± 178^{abcd}	148 ± 3^{a}	0.71 ± 0.01^{a}	3980 ± 411^{a}	55.9 ± 1.1^{ab}	0.47 ± 0.02^{a}	2.63 ± 0.40^{a}
		100%	7200 ± 379^{a}	147 ± 4^{a}	0.70 ± 0.02^{a}	3839 ± 297^{a}	56.3 ± 0.4^{ab}	0.46 ± 0.00^{a}	2.67 ± 0.17^{a}
	Gox	50%	7990 ± 247^{bcd}	146 ± 3^{a}	0.71 ± 0.00^{a}	4316 ± 161^{a}	55.4 ± 0.0^{ab}	0.45 ± 0.01^{a}	2.39 ± 0.02^{a}
		100%	7375 ± 0^{ab}	145 ± 4^{a}	0.73 ± 0.01^{a}	4027 ± 482^{a}	54.6 ± 0.4^{a}	0.46 ± 0.02^{a}	2.26 ± 0.11^{a}
	Xyl	50%	8502 ± 269^{d}	143 ± 3^{a}	0.69 ± 0.02^{a}	3894 ± 464^{a}	56.0 ± 0.1^{ab}	0.48 ± 0.02^{a}	2.68 ± 0.08^{a}
		100%	8077 ± 122^{bcd}	144 ± 4^{a}	0.70 ± 0.01^{a}	3969 ± 249^{a}	56.5 ± 0.1^{b}	0.46 ± 0.01^{a}	2.53 ± 0.12^{a}
		Average	**7887**	**147**	**0.70**	**4216**	**55.6**	**0.46**	**2.42**
		Min	**7200**	**143**	**0.69**	**3894**	**54.6**	**0.43**	**2.07**
		Max	**8502**	**153**	**0.71**	**5041**	**56.5**	**0.48**	**2.68**
CDC Plentiful	Null	-	7671 ± 131^{a}	149 ± 1^{a}	0.71 ± 0.03^{a}	4396 ± 330^{a}	55.0 ± 0.4^{a}	0.44 ± 0.02^{a}	2.20 ± 0.12^{a}
	AA	50%	8633 ± 351^{a}	148 ± 1^{a}	0.71 ± 0.01^{a}	4828 ± 187^{a}	55.3 ± 0.6^{a}	0.44 ± 0.01^{a}	2.26 ± 0.00^{b}
		100%	8296 ± 412^{a}	145 ± 2^{a}	0.70 ± 0.03^{a}	4850 ± 70^{a}	55.3 ± 0.1^{a}	0.43 ± 0.01^{a}	2.16 ± 0.11^{a}
	ADA	50%	7840 ± 115^{a}	145 ± 3^{a}	0.71 ± 0.01^{a}	4219 ± 459^{a}	55.2 ± 0.3^{a}	0.46 ± 0.03^{a}	2.34 ± 0.21^{ab}
		100%	7734 ± 361^{a}	146 ± 0^{a}	0.71 ± 0.00^{a}	4303 ± 369^{a}	55.8 ± 0.4^{a}	0.45 ± 0.01^{a}	2.49 ± 0.13^{ab}
	Gox	50%	8317 ± 47^{a}	145 ± 1^{a}	0.73 ± 0.00^{a}	4622 ± 647^{a}	55.0 ± 0.3^{a}	0.45 ± 0.03^{a}	2.31 ± 0.18^{ab}
		100%	8028 ± 317^{a}	147 ± 1^{a}	0.72 ± 0.01^{a}	4423 ± 500^{a}	55.1 ± 0.4^{a}	0.45 ± 0.01^{a}	2.37 ± 0.09^{ab}
	Xyl	50%	8270 ± 95^{a}	144 ± 4^{a}	0.70 ± 0.03^{a}	4584 ± 337^{a}	55.3 ± 0.1^{a}	0.45 ± 0.01^{a}	2.26 ± 0.07^{ab}
		100%	8256 ± 168^{a}	144 ± 4^{a}	0.70 ± 0.03^{a}	4423 ± 78^{a}	55.5 ± 0.1^{a}	0.45 ± 0.01^{a}	2.31 ± 0.03^{ab}
		Average	**8116**	**146**	**0.71**	**4516**	**55.3**	**0.45**	**2.30**
		Min	**7671**	**144**	**0.70**	**4219**	**55.0**	**0.43**	**2.16**
		Max	**8633**	**148**	**0.73**	**4850**	**55.5**	**0.46**	**2.49**

Table 5.2. Cont…

Cultivar	Additive	Conc.	Slice area (mm^2)	Slice brightness	Cell contrast	Number of cells	Area of cells (%)	Cell wall thickness (mm)	Cell diameter (mm)
Stettler	Null	-	7100 ± 15^{a}	146 ± 4.3^{a}	0.70 ± 0.01^{a}	3425 ± 279^{ab}	55.4 ± 0.0^{ab}	0.47 ± 0.02^{a}	2.50 ± 0.08^{a}
	AA	50%	7227 ± 129^{ab}	149 ± 3.0^{a}	0.72 ± 0.01^{a}	4569 ± 298^{bc}	54.2 ± 0.6^{a}	0.42 ± 0.00^{a}	2.01 ± 0.10^{a}
		100%	7806 ± 93^{b}	145 ± 0.7^{a}	0.71 ± 0.01^{a}	4683 ± 420^{c}	54.8 ± 0.1^{ab}	0.43 ± 0.02^{a}	2.08 ± 0.14^{a}
	ADA	50%	7695 ± 49^{ab}	146 ± 3.7^{a}	0.71 ± 0.01^{a}	4166 ± 57^{bcd}	55.5 ± 0.8^{ab}	0.45 ± 0.00^{a}	2.39 ± 0.12^{a}
		100%	7198 ± 30^{ab}	146 ± 1.9^{a}	0.71 ± 0.01^{a}	3391 ± 349^{abc}	56.0 ± 0.5^{ab}	0.48 ± 0.02^{a}	2.68 ± 0.30^{a}
	Gox	50%	7527 ± 252^{ab}	144 ± 1.2^{a}	0.71 ± 0.02^{a}	3791 ± 71^{abc}	55.3 ± 0.9^{ab}	0.47 ± 0.01^{a}	2.41 ± 0.18^{a}
		100%	7181 ± 195^{ab}	146 ± 2.6^{a}	0.72 ± 0.01^{a}	3338 ± 265^{a}	54.9 ± 0.2^{ab}	0.48 ± 0.02^{a}	2.54 ± 0.20^{a}
	Xyl	50%	7785 ± 55^{b}	141 ± 7.2^{a}	0.68 ± 0.02^{a}	3606 ± 420^{abc}	56.2 ± 0.0^{b}	0.48 ± 0.02^{a}	2.65 ± 0.22^{a}
		100%	7211 ± 309^{ab}	142 ± 5.1^{a}	0.69 ± 0.03^{a}	3661 ± 320^{abc}	55.8 ± 0.1^{ab}	0.46 ± 0.01^{a}	2.40 ± 0.01^{a}
		Average	**7414**	**145**	**0.71**	**3848**	**55.3**	**0.46**	**2.41**
		Min	**7100**	**141**	**0.68**	**3338**	**54.8**	**0.42**	**2.01**
		Max	**7806**	**149**	**0.72**	**4683**	**56.2**	**0.48**	**2.68**

Abbreviations: AA (ascorbic acid); L-Cys (L-cysteine); Gox (Glucose oxidase); ADA (Azodicarbonamide); and Xyl (Fungal Xylanase)
Data within the same colunm with different letters are significantly different ($p<0.05$).

5.5. Conclusion

The effectiveness of different additive types and levels was found to be highly cultivar specific. For instance, Glenn (stronger cultivar) had a better overall performance, even in comparison with the added oxidizers and/or enzymes regardless of the concentration. In contrast, Stettler considered to be an intermediate strength cultivar, had poorer overall performance in relation to other cultivars, with bread loaves having the lowest volumes, oven rise, and mixing time. Surprisingly, the addition of xylanase had a positive effect on flours with medium gluten strength, through improvement of loaf volume and crumb firmness. Considering the frequent implementation of dough improvers (oxidizers and enzymes) in the bakery industry, xylanase appeared to have a positive performance effect in baking in comparison to the oxidizers, resulting in equal or better performance for all cultivars.

5.6. Linkage

Due to the good overall grain and flour quality of all the five cultivars tested, in addition to similar or low improvement on bread quality and dough handling with the addition of enzyme or chemical oxidizers, a test with reducing agents was suggested. The next study was performed to understand how L-cysteine could or not improve dough development time in relation to rheology (i.e., dough strength) and final baking performance (i.e., loaf volume and crumb structure) of the same set of five cultivars based on their strength (i.e., from weak to strong). Thus, to determine if it could be beneficial to be added at process using higher dough strength, but needing shorter mixing time for bread production and maintaining the final product consistency and quality.

6. EFFECT OF L-CYSTEINE ON THE RHEOLOGY AND BAKING QUALITY OF DOUGHS FORMULATED WITH FLOUR FROM FIVE CONTRASTING CANADA SPRING WHEAT CULTIVARS[3]

6.1. Abstract

Wheat grain quality parameters are influenced by the composition of gluten proteins. To overcome wheat grain quality limitations, dough improvers such as reducing agents can be added to reduce mixing time and improve dough extensibility. The overall objective of this research was to examine the effect of L-cysteine (L-cys) concentration on the rheology and baking quality of doughs prepared using five western Canadian spring wheat cultivars. The addition of L-cys resulted in a significant (p<0.05) decrease in dough strength and handling properties, where stronger gluten strength wheats were less effected by addition and had improved dough handling properties, loaf volume, and softer crumb structure. The relationship between dough mixing time and bread quality is crucial for the baking industry. Therefore, reducing agents can be used in stronger wheat cultivars as means to improve efficiency of production (i.e., lower mixing time) and result in equal or higher quality bread loaf (i.e., loaf volume). The addition of L-cys to wheat flours reduced mixing time up to 47%, increased loaf volume (up to 9%), and elasticity of the products, those characteristics are desired to increase the efficiency of the automated processes for bread products.

Significance and novelty: The optimization of time versus quality of bread is crucial for the industry. Therefore, reducing agents can be used in stronger wheat cultivars as means to improve efficiency of production (i.e., lower mixing time) and result in equal or higher quality bread loaf (i.e., loaf volume).

6.2. Introduction

Bread wheat (*Triticum aestivum* L.) is the most widely consumed cereal grain and the second most-produced crop around the world (USDA, 2019). Unlike other cereal grains, the

[3] Tozatti, P., Fleitas, M.C., Briggs, C., Hucl, P., Chibbar, R.N., Nickerson, M.T. (2019). Effect of L-cysteine on the rheology and baking quality of doughs formulated with flour from five contrasting Canada spring wheat cultivars. *Cereal Chemistry*, 00, 13 November 2019: 1–13. https ://doi.org/10.1002/cche.10239.

viscoelastic properties of its gluten matrix allows it to be used to produce diverse baked products that are ethno-culturally determined in a wide range of baking applications. Those properties can be affected by different factors, such as genotype, environment, and management conditions (Bhatta et al., 2017). The moisture (precipitation) and nitrogen availability directly influence the metabolic activity in the plant, thus the synthesis and composition of protein in the kernel. For instance, conditions such as temperature, nutrient deficiencies, and water stress can reduce the grain-filling period (i.e., gliadin accumulates earlier in grain filling than glutenin), therefore increasing gliadin concentration and decreasing glutenin within a kernel (Park et al., 2014). Glutenin and gliadins are the main contributors to wheat quality representing 80% to 85% of the total flour protein and confer different properties of elasticity (glutenins) and extensibility (gliadins), (Anjum et al., 2007; Nadeem et al., 2015). Glutenins are heterogeneous polypeptides with molecular weights ranging from 12 to 130 kDa, and can be classified as high molecular weight glutenins (HMW-GS) and low molecular weight glutenins (LMW-GS). These polypeptides are present as polymers joined by disulfide cross-linkages (e.g., reaction with cysteine Cys-SH) to give dough elasticity. In contrast, gliadins are a heterogeneous mixture of monomeric proteins with molecular weights ranging from 30 to 80 kDa, capable of forming intramolecular disulphide bonds, to give the dough its extensibility (Anjum et al., 2007, Delcour and Hoseney, 2010). The HMW-GS are highly correlated with bread-making quality, LMW-GS and gliadins have significant effects on dough extensibility because they account for 75% of the total gluten (Khelifi and Branlard, 1992; Gupta and MacRitchie, 1994; He et al., 2005; Figueroa et al., 2009; Li et al., 2010). Similarly, analyses of five crosses made with New Zealand wheat genotypes revealed that glutenin proteins differentially affected wheat flour quality (Luo et al. 2001).

The transition of global population from rural to urban areas combined with changing lifestyle requires food products with ease of preparation, high and consistent quality to meet consumer preferences. Therefore, the baking industry is focused to improve the processing quality that will yield a uniform product consistingly meeting customer preference with minimum cost. Canadian wheat cultivars have properties that meet the specific end-use requirements (Canadian Grain Commission, 2019). However, processing and end-use quality are affected by the environment (Malik et al., 2013; Nehe et al., 2019), which can alter the flour attributes and alter the end-product quality. Dough improvers can alleviate flour quality deficiencies in bread-making, to consistently produce desirable quality end-productsbe to meet customer preferences. In baking industry, reducing agents are used as dough conditioners to decrease the mixing time and improve dough extensibility by reducing the average molecular

weight of glutenin protein aggregates. L-cysteine (L-cys) breaks down S-S bonds within the gluten network by converting them into SH groups (Wieser, 2003; Stoica et al., 2010). Usually, L-cys is used in concentrations ranging from 30 to 70 mg,L^{-1} and in the hydrochloride form, due to its solubility in water. An over-addition of this reducing agent can result in sticky and poor-handling doughs (Wieser, 2003).

The overall objective of this research was to examine the effect of L-cys concentration on the rheology and baking quality of doughs prepared using five Canadian wheat cultivars (Glenn, Harvest, Lillian, CDC Plentiful, and Stettler), varying in gluten strength (weak, intermediate and strong). We hypothesised that the reducing agent can have major impact in bread-making parameters in the intermediate and strong cultivars since they carry *Glu-A3d* (Glenn, strong) or *Glu-A3e* (Lillian, Plentiful and Stettler, intermediate).

6.2 MATERIALS AND METHODS

6.2.1. Materials

Five commercial grade Canadian spring wheat (Triticum aestivum L.) cultivars differing in gluten strength ranging from weak (cv. Harvest), to intermediate (cv. Lillian, CDC Plentiful and Stettler) and strong (cv. Glenn) were used in this study. All wheat cultivars were grown in a replicated trial during the 2017 crop year at the Kernen Crop Research Farm (52.158; -106.524; altitude 457m), University of Saskatchewan (Saskatoon, SK, Canada) on a Sutherland clay, clay-loam soil. The reducing agent L-cysteine hydrochloride was purchased from Sigma-Aldrich Co. (St Louis, MO, USA).

6.2.2. Grain meal and flour preparation

Grain (~5 g) from each sample was ground in a Thomas–Wiley laboratory grinder (Arthur H. Thomas Co., Philadelphia, PA, USA). To determine the dry weight, meal (~ 1 g) was placed in an oven set at 135 ^{o}C to air dry to constant weight (AACCI Approved Method 44-15.02). The determined grain moisture content was used to temper grain to 14.5% for ~18 h and then, milled into flour using a a Barbender Quadrumat Senior Experimental Mill (Brabender, South Hackensack, NJ, USA), as modified by Jeffers and Rubenthaler (1977).

6.2.3. Grain protein extraction and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The glutenins and gliadins were sequentially extracted from grain meal using the method of Singh et al (1991). Grain meal (20 mg) was extracted with propan-1-ol (50% v/v) at 65 ºC for 30 min with constant mixing using a Thermomixer (Eppendorf R, 1,400 rpm). The extraction mixture was centrifuged at 10,000 rpm for 2 min to separate the pellet containing the glutenins and the supernatant with the gliadins. Then, the supernatant was air dried overnight to concentrate the gliadins. The pellet was extracted with propan-1-ol (50% v/v), 0.08 M Tris-HCl, sodium dodecyl sulfate (SDS, 2% w/v), pH 8.0 and dithiothreitol (1.5% w/v) at 65 ºC with constant mixing for 30 min using a Thermomixer (EppendorfR , 1,400 rpm). The extraction mixture was centrifuged at 10,000 rpm for 2 min the glutenin polypeptides were treated with propan-1-ol (50% v/v), 0.08 M Tris-HCl, SDS (2% w/v), pH 8.0 and 4-vinyl pyridine (1.4% v/v) to alkylate the sulfhydryl (SH) groups, to separate the low molecular weight glutenin subunits. Both, the gliadin and glutenin polypeptides were denatured with SDS in the gel loading buffer. The denatured polypeptides were separated using denaturing polyacrylamide (15% w/v) gel electrophoresis (constant current 12.5 mA; 20 h; temperature 15 ºC). After electrophoresis, the separated polypeptides were visualized by staining with Coomassie blue (0.01% w/v). The nomenclature proposed by Payne and Lawrence (1983) was used for HMW-GS, whereas Gupta and Shepherd (1990), Jackson et al. (1996), Branlard et al. (2003) and Appelbee et al. (2009) were used for both, LMW-GS and ω-gliadins. The glutenin and gliadin polypeptide analyses were done using grain from three biological replicates.

6.2.4. Rheology analysis

Dough was prepared from the flour obtained from grain harvested from three independent biological replicates. Mixograph (10 g; TMCO National Mfg., Lincoln, NE) analysis was done following the AACCI Approved Method 54-40.02. The formulation used was based on the basic dough ingredients, such as flour (weight on a 14% m.b.), water (weight based on micro-doughLAB absorption), and NaCl (2.0% by weight). The micro-doughLAB absorption were 58.0% (Glenn), 60.7% (Harvest), 61.2 (Lillian), 59.8% (Stettler) and 57.8% (CDC Plentiful). The L-cys was added at two concentrations, representing 50% and 100% of the allowable limits for the baking industry from Health Canada (2012), where the limit for L-cys is 90 ppm (Health Canada, 2012). Each sample was mixed to peak dough development and allowed to rest for 60 min before being analyzed by rheological testing. In addition, for each

cultivar, dough without L-cys was prepared to be used as control. Dough for each treatment was prepared in with flour from each of the three biological replicate.

The small amplitude shear rheometry was performed based on the method of Jekle and Becker (2011) using an AR-2000 rheometer (TA Instruments, New Castle, DE, USA) utilizing a 40 mm parallel plate fixture. The method consisted in a dough sample (~ 5 g) that was placed between the parallel plates where one of them was lowered to a 2 mm gap, and the excess of dough was removed. To ensure that the sample would not dry out, paraffin oil was added to the dough surface. The dough was rested for approximately 10 min and the temperature was kept constant at room temperature (21-23 °C) until the end of the experiment. Parameters such as the dynamic storage (G′), loss (G″), complex (|G*|) and loss tangent were determined as a function of frequency (0.1-100 Hz) at a constant amplitude strain of 0.1%. Values at a frequency of 1 Hz were arbitrary selected to compare the cultivars. After the oscillatory frequency test, creep recovery was determined on the same dough sample at a constant shear stress (τ_0 = 250 Pa) for 180 s. After the stress was removed, the relaxation of the dough was observed for 360 s. As a function of time, the strain values were recorded and evaluated with the following equation:

$$J(t) = \gamma(t)\tau_0^{-1} \quad \text{(Eq. 1)}$$

where J is the compliance (Pa^{-1}), t is the time (s), γ is the strain, and τ_0 is the stress (constant) applied during test. The creep compliance J_{max} (at t = 180 s of the creep phase), and the relative elasticity of the sample (J_{el}) were determined, based on the mechanical energy stored in the dough sample, J_r (t= 360 s in the recovery phase), by the following equation (Eq. 2):

$$J_{el} = J_r(J_{max})^{-1} \quad \text{(Eq. 2)}$$

All oscillatory rheology measurements were made within the linear viscoelastic regime, however, the creep compliance was not. Samples were analysed in triplicates (n = 3) and the data was presented as mean ± standard deviation.

6.2.5. Bread-making

A short fermentation method (Canadian Short Process - CSP) described by the Canadian Grain Commission (2016) was used for this study. The bread dough formulation contained 100 g of wheat flour (14.0% moisture basis, m.b.), 2 g salt, 3 g of shortening (Crisco all-vegetable

shortening), 4 g sugar, 3 g fresh compressed yeast (Fleishman compressed yeast), and optimum water (based on farinograph analysis, FAB). The water absorption (FAB) was assessed for each cultivar by the AACCI Approved Method 54–21 (C.W. Brabender Instruments, Inc., South Hackensack, NJ, USA). The FAB values were 61.7% (Harvest), 63.2% (Lillian), 61.4% (Glenn), 59.5% (Stettler) and 60.6% (CDC Plentiful). L-cys at the concentration of 50% (45 ppm) and 100% (90 ppm) of the allowable inclusion concentrations specified from Health Canada (2012). For consistent distribution, the reducing agent was added to each dough as a solution. The bread dough ingredients were mixed to slightly past peak in a Swanson Mixer (National Manufacturing Co., Lincoln, NE, USA) at 165 rpm, and peak mixing time (min) and the mixing energy (Wh/kg) was recorded. After mixing, the dough was rounded by hand and placed in a fermentation cabinet (National Manufacturing Co., Lincoln, NE, USA) at 34°C and 85% RH (relative humidity). Each dough was punched by hand at 15 min, allowed to proof for further 15 min and then panned at 30 min. The proofing time was determined by using the time to reach 9 cm proof for baking controls, this time was used for all the baking doughs (~60 min, usually). Loaves were baked after proofing time for 22 min at 400 ºF (205 ºC) (Reel Type Oven, National Manufacturing Co., Lincoln, NE, USA). For each treatment cultivar-concentration, two loaves were baked.

6.2.6. Bread quality

The oven rise (cm) was determined by the difference between the loaf height before and after baking. Each loaf was cooled at room temperature (21-23 ºC) for 60 min. Then, the loaf volume (LV, cm^3) was measured using the rapeseed displacement method with a National Loaf Volumeter (National Manufacturing Company, Lincoln, NB, USA) determined according to the AACCI Approved Method 10-05.01 and Cathcart and Cole (1938). Each bread loaf was allowed to cool at room temperature and placed into plastic bags for further crumb structure analysis and C-cell imaging crumb analysis (~24 h). The bread crumb firmness was determined on individual ~2.54 cm slices of bread using a TA.XT plus Texture Analyser (Stable Microsystems, Surrey, UK), following the Approved Method AACCI 74-09.01. In summary, a flat ended cylindrical probe of 36 mm in diameter was pushed into the bread at a speed of 2 $mm.s^{-1}$ to a total distance of 8 mm and the force was recorded (gram force, gF). Data was presented as mean and standard deviation from three slices of each of the two separate loaves.

A C-cell monochrome (CC.300) imaging system (Calibre Control International, name of city, UK) was used to characterize the crumb structure of the bread, following the AACC International method 10-18.01. Slices of one inch (~2.54 cm) were cut using an electric knife

(Hamilton Beach 74275RC), then the middle slice was again cut in ½ inch to be analyzed in the C-cell equipment. Usually, the C-Cell monochrome system generates 48 numerical results, from those, only seven were focused in this study, such slice area (mm^2), slice brightness (unitless), cell contrast (unitless), number of cells, area of cells (%), cell wall thickness (mm), and cell diameter (mm). Data was presented as mean and standard deviation from two separate loaves for each cultivar, additive type, and different concentrations (50 and 100%).

6.2.7. Statistical analysis

A 2-way analysis of variance (ANOVA) analysis was performed to test the significance of the main effects (i.e., cultivar-type and or additive-concentration) and 2-way interactions (i.e., cultivar × additive-concentration). In addition, a one-way ANOVA with a Tukey Post-Hoc test were performed within each cultivar to show differences between additive and concentration. In addition, Pearson correlations were made relating baking parameters and bread loaf properties. All statistical analysis was performed using SPSS Grad Pack v24 software. A one-way ANOVA was also performed on loaf volume data collected for the baking controls over multiple days/weeks to prove that there was no 'day effect' occurring during the baking studies.

6.3. Results and discussion

6.3.1. Composition of high and low molecular weight glutenin, and ω-gliadins subunits

The HMW-GS, the LMW-GS and the ω-gliadins alleles identified from the five wheat cultivars are presented in Table 6.1.

Table 6.1. Allelic composition at loci *Glu-A1*, *Glu-B1*, *Glu-D1* (encoding HMW-GS), *Glu-A3*, *Glu-B3*, *Glu-D3* (LMW-GS) and *Gli-B1* (ω-gliadins) five Canadian spring wheat cultivars (2017 crop year).

Genotype	Entry	Crude Protein[1]	HMW			LMW			ω-Gliadins
			Glu-A1	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	*Gli-B1*
Harvest	1	13.1	2*	7+9	5+10	f	h	c	d
Glenn	2	14.0	2*	7+9	5+10	d	h	c	d
Lillian	3	13.4	2*	7oe+8	5+10	e	h	c	d
CDC Plentiful	4	12.1	1	7oe+8	5+10	e	h	c	d
Stettler	5	14.1	2*	7+9	5+10	e	h	a	d

[1] Results at 14% moisture basis (m.b.)

Based on their mobility on SDS-PAGE (Figure 6.1), at the *Glu-A1* locus two allelic variations were observed: subunit 2* was the most common polypeptide present in four cultivars, whereas the subunit 1 was present in CDC Plentiful. At *Glu-B1*, two allelic variations were found: 7+9 was carried by three cultivars (60%) and 7oe+8 was present in Lillian and CDC Plentiful. The HMW-GS *Glu-D1* did not show allelic diversity as all five cultivars carried the 5+10 allele (Table 6.1).

Most of the cultivars from the Canadian Western Red Spring (CWRS) market class have the glutenin subunits composition *Glu-A1* 2*, *Glu-B1* 7+9 and *Glu-D1* 5+10 (Bushuk, 1998; Bekes et al., 2007) known as desirable alleles since they confer superior processing quality.

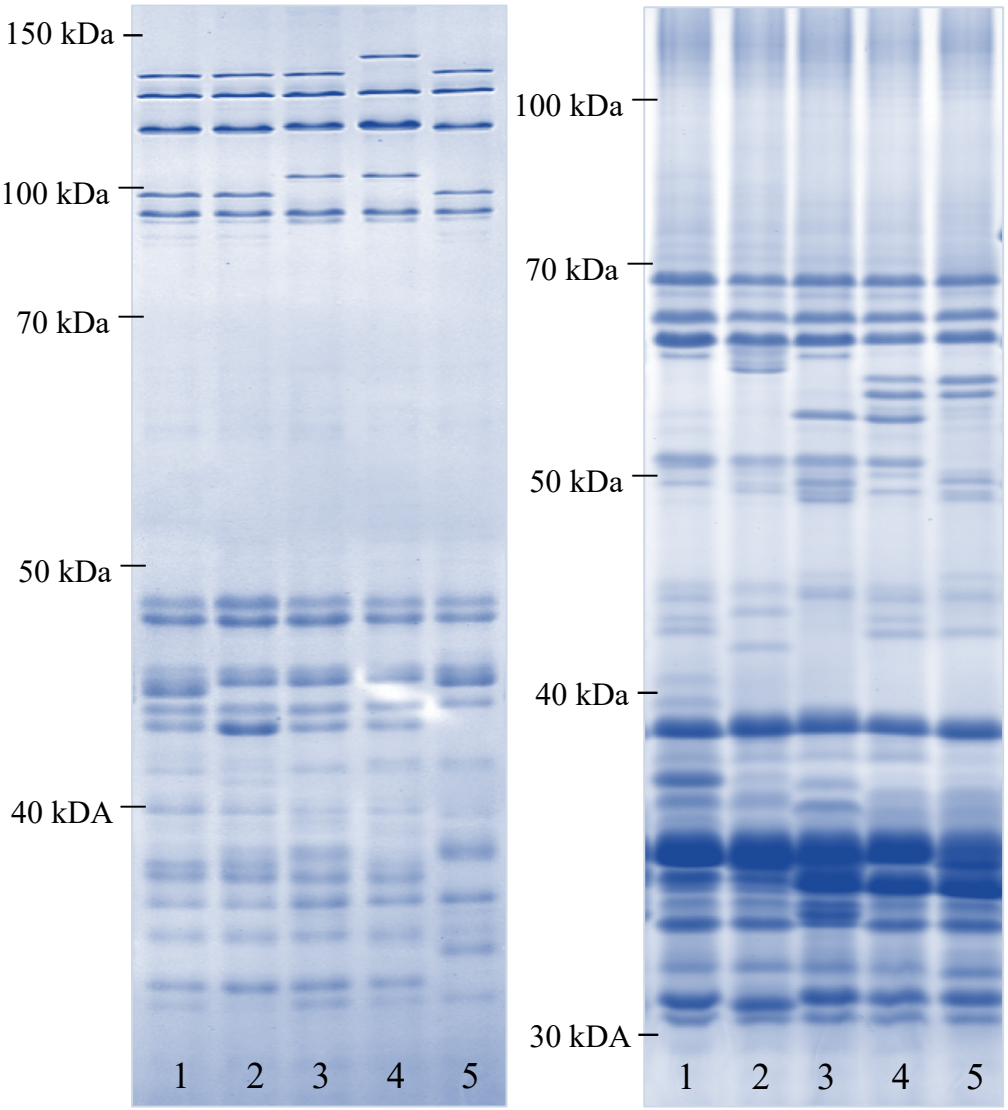

Figure 6.1. SDS-PAGE of five Canadian spring wheat cultivars (2017 crop year), showing glutenins (left) and gliadins (right) protein banding patterns. 1: Harvest, 2: Glenn, 3: Lillian, 4: CDC Plentiful and 5: Stettler.

For instance, several studies have reported statistically better effects of the alleles 1 and 2* on all quality parameters than the null allele of the *Glu-A1* locus (Sontag-Strohm et al., 1996; He et al., 2005; Liu et al., 2010). The positive effects *Glu-B1* locus are also true for the subunits 7+8, and 7+9 compared to the 6+8 (Figueroa et al. 2009; Zhang et al. 2009). The subunit 7oe+8 confered improved dough strength (Butow et al., 2003; Shewry et al., 2007; Bekes et al., 2007). The glutenin subunits 8 and 9 were strongly associated with desirable rheological and bread-making quality parameters (Khan et al., 1990).

The superior effects on quality parameters of the *Glu-D1* locus, subunit 5+10 compared to the 2+12 subunit was usually ascribed to the presence of an extra cysteine residue in the Dx-5 compared to the Dx-2 subunit (Payne, 1987; Luo et al., 2001; Liu et al., 2005; Shewry et al., 2007), which promoted the formation of polymers with larger size distribution (Ikeda et al., 2007). The *Gli-B1* locus encoding the ω-gliadins did not show allelic diversity, as all the five cultivars carried the *d* polypeptide (Table 6.1), the predominant subunit in Canadian western red spring wheat cultivars (Metakovsky et al., 2007; 2018), and associated with better dough quality (Branlard and Metakovsky, 2007).

6.3.2. Rheology

A 2-way ANOVA analyses of rheology results for the complex shear modulus (|G*|), the loss tangent (tan δ), the maximum compliance (J_{max}) and relative elasticity (J_{el}) for all dough treatments determined that all main effects for each rheological parameter were statistically significant (p<0.001) (Table 6.2). The 2-way interactions between cultivar-type and L-cys concentration was significant (p<0.001) only for |G*| and J_{max}, but not for tan δ and J_{el} (p>0.05). In all cases, as the L-cys concentration increased, there was a reduction in |G*|, however the magnitude of reduction differed between cultivars (Figure 6.2A).

Table 6.2. Concentration effect of L-cysteine on dough rheology of five Canadian spring wheat cultivars (2017 crop year). Data represents the mean of replicate measurements ± one standard deviation (n=3).

Cultivar	L-Cys (%)	tan δ (-)	|G*| (kPa)	J_{max} (mPa^{-1})	J_{el} (-)
Harvest	0	0.33 ± 0.01^{a}	13.7 ± 0.3^{c}	0.99 ± 0.50^{a}	0.57 ± 0.23^{b}
	50	0.39 ± 0.01^{b}	8.1 ± 0.2^{b}	3.22 ± 0.09^{b}	0.50 ± 0.01^{b}
	100	0.45 ± 0.01^{c}	5.6 ± 1.1^{a}	14.39 ± 1.19^{c}	0.16 ± 0.02^{a}
Glenn	0	0.32 ± 0.01^{a}	19.1 ± 0.9^{c}	0.34 ± 0.06^{a}	0.75 ± 0.01^{b}
	50	0.34 ± 0.01^{a}	15.6 ± 0.9^{b}	0.53 ± 0.10^{a}	0.72 ± 0.02^{b}
	100	0.43 ± 0.07^{b}	8.6 ± 0.4^{a}	3.37 ± 0.74^{b}	0.47 ± 0.05^{a}
Lillian	0	0.35 ± 0.01^{a}	17.6 ± 1.4^{b}	0.50 ± 0.08^{a}	0.72 ± 0.01^{c}
	50	0.43 ± 0.01^{b}	8.6 ± 1.3^{a}	2.67 ± 1.04^{a}	0.55 ± 0.08^{b}
	100	0.47 ± 0.04^{c}	7.4 ± 0.8^{a}	9.55 ± 1.20^{b}	0.24 ± 0.02^{a}
CDC Plentiful	0	0.34 ± 0.01^{a}	20.3 ± 1.1^{c}	0.37 ± 0.03^{a}	0.72 ± 0.02^{b}
	50	0.39 ± 0.01^{b}	11.6 ± 0.5^{b}	1.25 ± 0.26^{b}	0.67 ± 0.04^{b}
	100	0.44 ± 0.01^{c}	8.2 ± 0.4^{a}	3.79 ± 0.50^{c}	0.46 ± 0.04^{a}
Stettler	0	0.35 ± 0.00^{a}	11.6 ± 0.7^{b}	0.75 ± 0.07^{a}	0.73 ± 0.00^{c}
	50	0.41 ± 0.01^{b}	8.4 ± 1.2^{a}	2.62 ± 0.84^{a}	0.57 ± 0.06^{b}
	100	0.47 ± 0.01^{c}	6.8 ± 0.4^{a}	6.41 ± 1.27^{b}	0.34 ± 0.06^{a}

Same letters within the same column and for the same cultivar, do not statistically differ ($p<0.05$)

It was assumed this reduction in strength was the result of reduced size of the glutenin aggregates, and the differences seen between cultivars relates to the spatial availablility of L-cys to the disulfide bonds. CDC Plentiful was the most affected by the concentration, with values being reduced to 20.3 kPa for the control to 8.2 kPa at the 100% level. The L-cys addition increased extensible properties of dough, which impacted the storage modulus (G′) being higher when cysteine was added, and also impacted the |G*| (Pecivová et al., 2010). The lowest reduction was for cv. Stettler, where it was reduced from 6.8 kPa (at 100%) from 11.6 kPa (control) (Figure 6.2A). Harvest had the highest J_{max} value at 100% concentration (14.29 mPa^{-1}), followed by Lillian (9.55 mPa^{-1}), Stettler (6.41 mPa^{-1}), CDC Plentiful (3.79 mPa^{-1}), and finally Glenn (3.37 mPa^{-1}) (Figure 6.2B). Settler showed an increase from the control (0.75 mPa^{-1}) to 2.62 mPa^{-1} at the 50% level, with further increment at 100% level (6.41. mPa^{-1}).

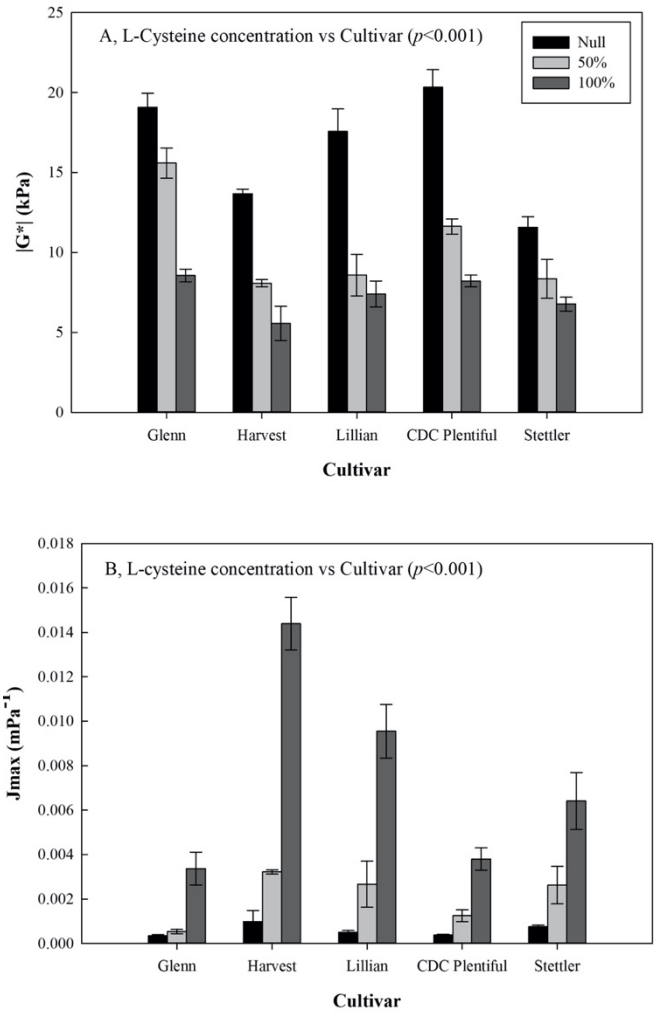

Figure 6.2. The effect of L-cysteine (p<0.001) and wheat cultivar-type on the complex modulus (|G*|) (kPa) (A) and Jmax (mPa^{-1}) (B) at 0%, 50%, and 100% concentration. Data represent the mean ± one standard deviation. *Abbreviations*: Null (no additive). Treatment concentration refers to 50 and 100% of the maximum permitted by Health Canada in foods for chemical additives.

The main effects for tan δ and J_{el} (Figure 6.3) for concentration and cultivar (p<0.001) showed that tan δ values were higher for cv. Lillian and Stettler, followed by Harvest, CDC Plentiful and Glenn (Figure 6.*3A*). Cultivar also had a significant effect on J_{el}, where Glenn had the highest values, followed by CDC Plentiful, Lillian, Stettler, and Harvest (Figure 6.3B). In relation to the concentration, tan δ (Figure 6.3C) increased and J_{el} decreased (Figure 6.3D) with increasing L-cys concentration. As J_{el} measured the relative elasticity, it was expected that cultivars (such as Glenn) with strong gluten strength to have higher values and less affected by reducing agents than cultivars (such as Harvest) with weak gluten strength, as they are proposed to have more disulfide linkages.

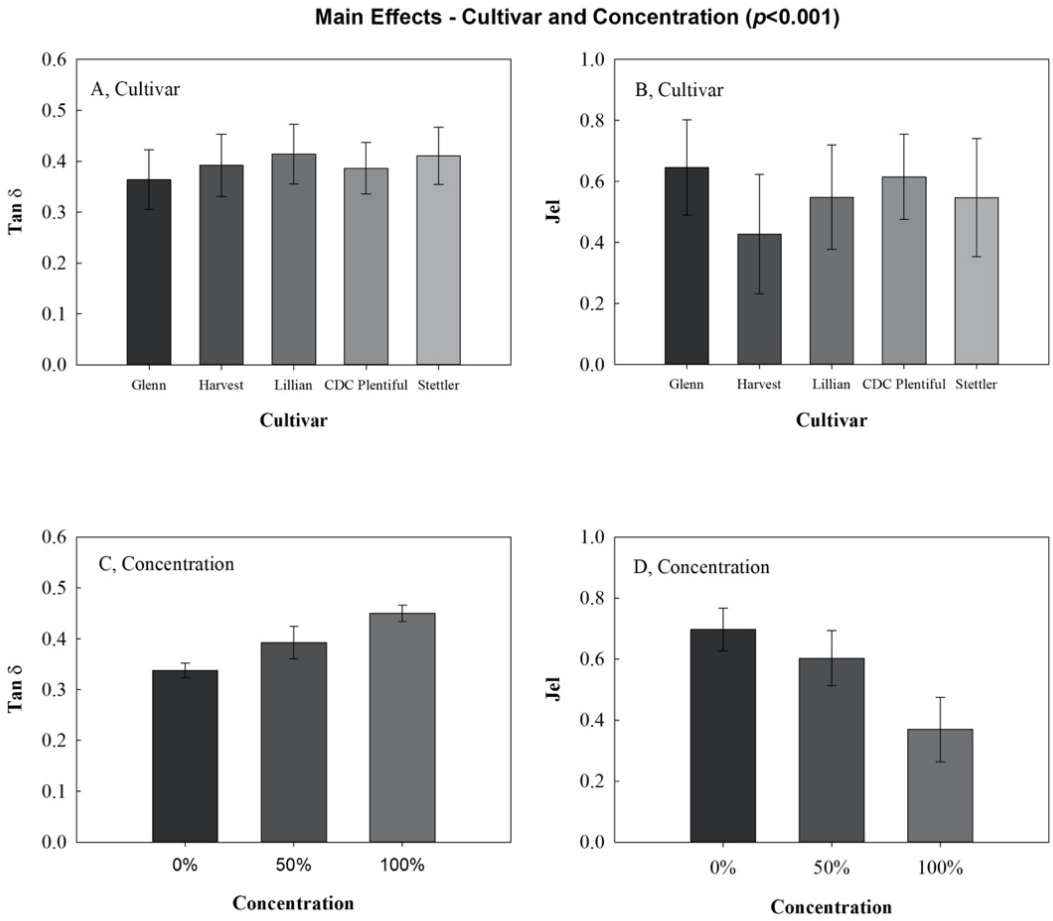

Figure 6.3. The effect of L-cysteine (p<0.001) for cultivar-type and concentration on the tan δ (A) and J_{el} (B) and L-cysteine concentration 0%, 50%, and 100% on the tan δ (C) and J_{el} (D). Data represent the mean ± one standard deviation. *Abbreviations*: Null (no additive). Treatment concentration refers to 50 and 100% of the maximum permitted by Health Canada in foods for chemical additives.

These findings suggested that the addition of L-cys led to a reduction in dough strength. Stoica et al. (2010) found that the addition of L-cys decreased dough elasticity, but increased adhesiveness, extensibility and machinability. The reduction in strength with increasing L-cys levels could result from the reduction in crosslinking within the gluten matrix, that reduced the size of aggregates and the formation of free SH groups (Song and Zheng, 2007). The free SH directly influenced the rheological properties and the performance of chemical improvers (Andrews et al., 1995).

6.3.3. Baking quality

A 2-way ANOVA for mixing time, oven rise, loaf volume, and crumb firmness parameters for all treatments found that the main effects (i.e., cultivar and concentration) to be highly significant (p<0.001) (with the exception of oven rise – concentration, p<0.01) (Table 3). The 2-way interaction was significant for mixing time (p<0.001) and loaf volume (p<0.05),

but not for oven rise and crumb firmness (p>0.05). Cultivar significantly affected mixing time with the addition of L-cys at both concentrations. However, overall values were reduced for all the five cultivars as the size of the glutenin aggreagates was presumed to be reduced (Figure 6.4A). A reduced mixing time can be beneficial in saving bread production time and energy cost (Wieser, 2003; Stoica et al., 2010).

On the other hand, excessive protein breakdown can negatively affect the bread dough, resulting in higher dough stickiness and poor handling capacity (Wieser, 2003). For cv. Glenn, the mixing time was reduced from 6.1 min (control) to 3.4 min (100% L-cys) concentration (Figure 6.4A). The cv. Harvest (with a weaker dough strength), showed the least variation (1.2 min) between control and 100% L-cys compared to cv. Lillian (1.8 min), CDC Plentiful (1.6 min) and Stettler (1.7 min).

Table 6.3. Concentration effect of L-cysteine on baking parameters of five Canadian spring wheat cultivars (2017 crop year). Data represents the mean of replicate measurements ± one standard deviation (n=2).

Cultivar	L-Cys (%)	Mixing Time (min)	Oven Rise (cm)	Loaf Volume (cm^3)	Crumb Firmness (gF)
Harvest	0	3.8 ± 0.2^{b}	2.4 ± 0.1^{b}	1023 ± 25^{b}	139 ± 11^{a}
	50	2.9 ± 0.2^{a}	2.4 ± 0.2^{b}	988 ± 4^{b}	156 ± 13^{a}
	100	2.6 ± 0.1^{a}	1.6 ± 0.1^{a}	923 ± 4^{a}	184 ± 8^{a}
Glenn	0	6.1 ± 0.3^{b}	3.3 ± 0.4^{a}	1148 ± 4^{a}	115 ± 21^{a}
	50	3.8 ± 0.1^{a}	3.7 ± 0.1^{a}	1250 ± 0^{a}	92 ± 8^{a}
	100	3.4 ± 0.2^{a}	3.0 ± 0.9^{a}	1105 ± 71^{a}	108 ± 12^{a}
Lillian	0	4.1 ± 0.1^{b}	2.4 ± 0.2^{a}	1053 ± 4^{b}	127 ± 10^{a}
	50	2.6 ± 0.1^{a}	2.7 ± 0.1^{a}	1068 ± 25^{b}	120 ± 4^{a}
	100	2.3 ± 0.1^{a}	1.8 ± 0.5^{a}	938 ± 4^{a}	173 ± 31^{a}
CDC Plentiful	0	4.2 ± 0.1^{b}	2.6 ± 0.0^{a}	1053 ± 4^{ab}	158 ± 15^{a}
	50	2.8 ± 0.0^{a}	3.0 ± 0.0^{b}	1083 ± 18^{b}	141 ± 1^{a}
	100	2.6 ± 0.3^{a}	2.7 ± 0.1^{ab}	1010 ± 0^{a}	172 ± 6^{a}
Stettler	0	4.3 ± 0.0^{b}	1.9 ± 0.1^{a}	960 ± 21^{b}	180 ± 12^{a}
	50	3.1 ± 0.0^{a}	2.0 ± 0.1^{a}	960 ± 14^{b}	173 ± 13^{a}
	100	2.6 ± 0.2^{a}	1.5 ± 0.7^{a}	860 ± 14^{a}	230 ± 1^{b}

Same letters within the same column and for the same cultivar, do not statistically differ (p<0.05)

Therefore, except for Glenn, samples had their mixing time reduced by approximately one-thirdwith the addition of 50% L-cys and showed a further time reduction with the increased L-cys concentration to 100% (Table 2). In addition, mixing time had a statistically significant positive correlation with loaf volume ($r = 0.49$, $p<0.01$) and |G*| ($r = 0.46$, $p<0.01$) (Table A6.2 and A6.3, Appendix).

The loaf volume of cv. Glenn showed a slight increase with the 50% L-cys, whereas all other cultivars did not differ from their respective controls (Figure 6.4B). However, as the L-cys concentration was increased to 100%, a reduction in loaf volume relative to the control was observed for all cultivars (Figure 6.4B).

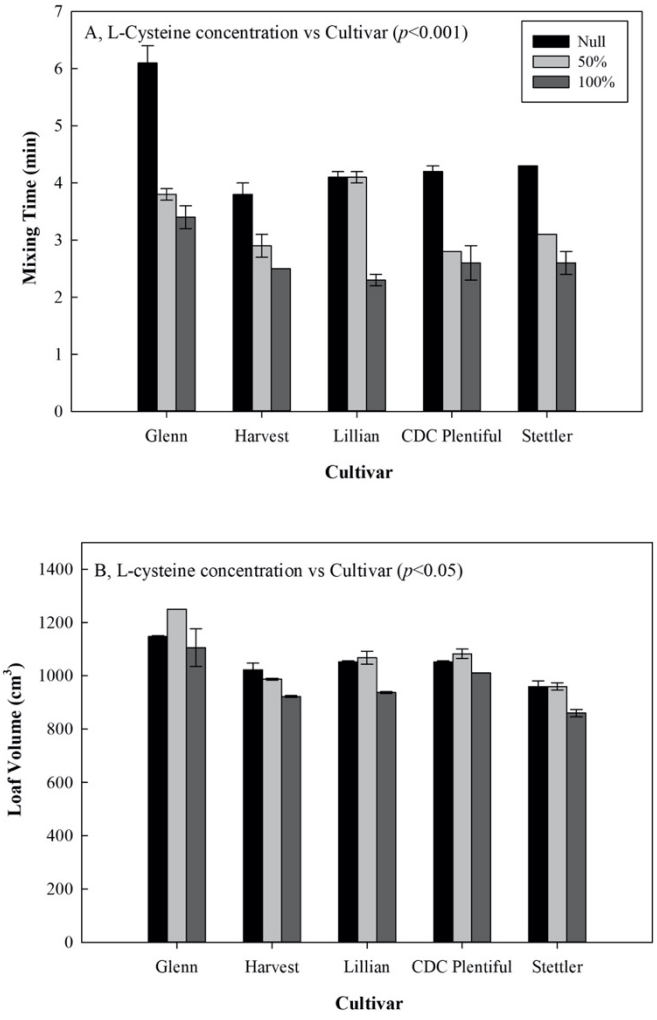

Figure 6.4. The effect of L-cysteine ($p<0.01$) and wheat cultivar-type on the mixing time (min) (kPa) (A) and on the loaf volume (cm^3) (B) at 0%, 50%, and 100% concentration. Data represent the mean ± one standard deviation. *Abbreviations*: Null (no additive). Treatment concentration refers to 50 and 100% of the maximum permitted by Health Canada in foods for chemical additives.

Loaf volume was overall greatest for Glenn, followed by Lillian and CDC Plentiful, and then Harvest and Stettler. In dough with strong gluten, L-cysteine addition increased the loaf volume of in the North American cultivars types, since prior to baking, the gas trapped within the dough developed a spongier dough (Weiser, 2003). Stoica et al. (2010) measured the volume of loaves with the addition of different L-cys concentrations (from 0 to 90 ppm) and observed an increase in loaf volume from 0-70 ppm addition, where 70 ppm had the highest volume (10% higher than the control). In the same study, the further addition of L-cys (90 ppm) decreased the loaf volume considerably, and it was similar to the control. A similar trend was found in this study. The negative effect of an increase in L-cys concentration can be attributed as a result of diminishing the dough ability to retain the fermentation gases (Stoica et al., 2010). In addition, positive and significant correlation was found between loaf volume and oven rise (r = 0.91, p<0.01), |G*| (r = 0.55, p<0.01), and J_{el} (r = 0.55, p<0.01) (Table A6.3, Appendix). However, there was a negative correlation with crumb firmness (r = -0.89, p<0.01) and tan δ (r = -0.54, p<0.01).

In the case of oven rise, cv. Glenn was the highest, followed by cvs. CDC Plentiful, Lillian, Harvest and Stettler (Figure 6.5A). In contrast, crumb firmness followed the opposite trend as a function of cultivar (Figure 6.5B). These findings suggest that cultivars that formed stronger dough, experienced greater rising, and resulted in a weaker crumb network. Weaker doughs rose less and formed stronger crumbs due to their increased density. Oven rise also saw an increase as L-cys concentrations were raised from 0 to 50% of the permitted concentration, and then declined once at the 100% of allowed concentration (Figure 6.5C). Crumb firmness was found to be similar at the 0 and 50% concentration, and then increased as it reached the maximum allowed concentration (100%) level since the loaves were denser in nature (Figure 6.5D). The addition of L-cys contributed to increased water absorption, therefore in lower concentrations (50%), loaf compressibility decreased indicating softer crumb. Whereas, with a further increase in L-cys concentration the compressibility increased resulting in decreased crumb softness (i.e., higher crumb firmness values) (Elkhalifa and El-Tinay, 2002; Pecivová et al., 2010). Negative and significant correlation was found between oven rise and crumb firmness (r = -0.71, p<0.01) and tan δ (r = -0.51, p<0.01). On the other hand, oven rise positively correlated with |G*| (r = 0.51, p<0.01) and J_{el} (r = 0.43, p<0.05).

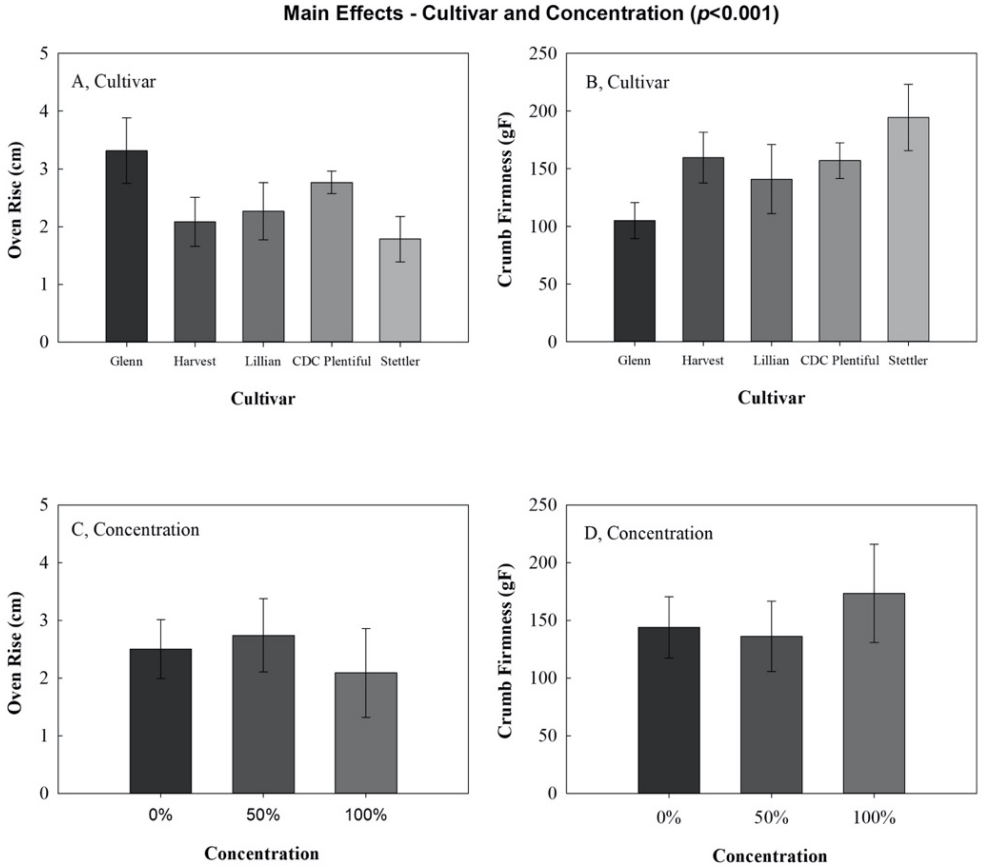

Figure 6.5. The effect of L-cysteine (p<0.001) for cultivar-type and concentration on the oven rise (cm) (A) and on the crumb firmness (gF) (B); and L-cysteine concentration 0%, 50%, and 100% on the oven rise (cm) (C) and on the crumb firmness (gF) (D). Data represent the mean ± one standard deviation. Treatment concentration refers to 50 and 100% of the maximum permitted by Health Canada in foods for chemical additives.

6.3.4. C-cell imaging and baking parameters

A 2-way ANOVA of the crumb parameters for all concentrations analyzed by C-cell imaging showed that the 2-way interaction cultivar x concentration was not statistically significant for all the C-cell parameters (p>0.05), as well as all main effects for cell contrast (p>0.05) (Table 4). Slice area had significant main effects interaction of cultivar by additive-concentration (p<0.001) (Table A6.1, Appendix). It was also positively correlated with oven rise (r = 0.91, p<0.01) and loaf volume (r = 0.98, p<0.01) and, negatively correlated to crumb firmness (r = -0.85, p<0.01) (Table A6.2, Appendix). The ANOVA for the number of cells indicated significant main effects of cultivar and additive-concentration (p<0.001). Number of cells was positively and significantly correlated with mixing time (r = 0.55, p<0.01), oven rise (r = 0.82, p<0.01), loaf volume (r = 0.85, p<0.01), and negatively correlated to crumb firmness (r = -0.74, p<0.01). Cell wall thickness correlated significantly and negatively to all baking parameters, such as mixing time (r = -0.54, p<0.01), oven rise (r = -0.55, p<0.01), loaf volume (r = -0.56, p<0.01) and crumb firmness (r = -0.53, p<0.01). Even though other parameters such

as slice brightness, area of cells, cell diameter had some significant two-way interactions of cultivar by additive-concentration (Table A6.1, Appendix), they were considered minor and did not strongly correlate with the baking attributes.

The variation in bread-making quality among different cultivars can be explained by the LMW glutenins (and in a smaller proportion gliadins) and their interactions with the HMW-GS, which play an important role in the determination of gluten strength and bread-making quality. In the present study, three allelic variations were observed at the *Glu-A3* locus. Thus, the null allele *Glu-A3e* was the most frequent (present in three cultivars) followed by the subunits *Glu-A3f*, present in cv. Harvest considered to have a weak gluten strength, and *Glu-A3d* carried by Glenn considered as strong. Furthermore, the cultivars under study did not show allelic diversity of LMW-GS *Glu-B3* as evidenced by the fact that all five cultivars carried the allele *Glu-B3h* (Table 1). The proportion of bands that make up the different alleles revealed in SDS-PAGE (Figure 6.1) showed a clear majority of the subunit *Glu-D3c* (four cultivars) followed by *Glu-D3a* present only in cv. Stettler (Table 6.1). Liu et al. (2005) studying the allelic variation at the *Glu-1* and *Glu-3* loci effect on mixographic properties in Chinese bread wheats found that all cultivars showing outstanding bread-making quality carried the *Glu-A3d* allele as well as *Glu-D1* 5+10. Despite the null allele (*e*) at the *Glu-A3* being usually associated with inferior quality parameters this could be compensated with excessive strength of the HMW glutenins subunits.

Table 6.4. Concentration effect of L-cysteine on C-cell parameters of five Canadian spring wheat cultivars (2017 crop year). Data represents the mean of replicate measurements ± one standard deviation (n=2).

Cultivar	Concentration (%)	Slice Area (mm^2)	Slice Brightness (-)	Cell Contrast (-)	Number of Cells (-)	Area of Cells (%)	Cell Wall Thickness (mm)	Cell Diameter (mm)
Harvest	0	7361 ± 161^{b}	148 ± 0^{b}	0.72 ± 0.00^{a}	3997 ± 44^{a}	55.4 ± 0.4^{a}	0.45 ± 0.01^{a}	2.30 ± 0.19^{a}
	50	7035 ± 134^{ab}	144 ± 2^{ab}	0.71 ± 0.01^{a}	3657 ± 392^{a}	55.8 ± 0.6^{a}	0.46 ± 0.02^{a}	2.47 ± 0.19^{a}
	100	6608 ± 30^{a}	136 ± 4^{a}	0.67 ± 0.02^{a}	2999 ± 146^{a}	56.8 ± 0.8^{a}	0.49 ± 0.02^{a}	2.56 ± 0.31^{a}
Glenn	0	8130 ± 46^{a}	146 ± 1^{a}	0.70 ± 0.02^{a}	4420 ± 157^{a}	55.4 ± 0.3^{a}	0.45 ± 0.01^{a}	2.31 ± 0.01^{a}
	50	8923 ± 93^{a}	144 ± 2^{a}	0.71 ± 0.00^{a}	4701 ± 9^{a}	56.6 ± 0.2^{a}	0.45 ± 0.00^{a}	2.52 ± 0.04^{a}
	100	7869 ± 709^{a}	147 ± 3^{a}	0.71 ± 0.01^{a}	4208 ± 354^{a}	55.9 ± 1.0^{a}	0.46 ± 0.00^{a}	2.54 ± 0.32^{a}
Lillian	0	7625 ± 9^{b}	149 ± 1^{a}	0.70 ± 0.03^{a}	4110 ± 372^{a}	55.5 ± 0.1^{a}	0.45 ± 0.02^{a}	2.39 ± 0.14^{a}
	50	7534 ± 74^{b}	144 ± 5^{a}	0.67 ± 0.05^{a}	3647 ± 501^{a}	57.0 ± 1.3^{a}	0.47 ± 0.03^{a}	2.86 ± 0.46^{a}
	100	6795 ± 37^{a}	144 ± 3^{a}	0.68 ± 0.00^{a}	3041 ± 397^{a}	56.7 ± 0.1^{a}	0.49 ± 0.02^{a}	2.86 ± 0.24^{a}
CDC Plentiful	0	7671 ± 131^{a}	149 ± 1^{a}	0.71 ± 0.02^{a}	4396 ± 330^{a}	55.0 ± 0.4^{a}	0.44 ± 0.01^{a}	2.20 ± 0.12^{a}
	50	7861 ± 246^{a}	150 ± 1^{a}	0.72 ± 0.00^{a}	4220 ± 50^{a}	55.3 ± 0.3^{a}	0.46 ± 0.00^{a}	2.54 ± 0.06^{b}
	100	7387 ± 239^{a}	146 ± 2^{a}	0.69 ± 0.01^{a}	3864 ± 173^{a}	55.9 ± 0.2^{a}	0.46 ± 0.01^{a}	2.67 ± 0.03^{b}
Stettler	0	7100 ± 15^{b}	145 ± 4^{a}	0.69 ± 0.01^{a}	3424 ± 279^{a}	55.4 ± 0.0^{a}	0.47 ± 0.02^{a}	2.50 ± 0.08^{a}
	50	7131 ± 226^{b}	141 ± 1^{a}	0.70 ± 0.01^{a}	3608 ± 334^{a}	55.6 ± 0.6^{a}	0.47 ± 0.02^{a}	2.49 ± 0.29^{a}
	100	6328 ± 36^{a}	140 ± 4^{a}	0.68 ± 0.02^{a}	2872 ± 222^{a}	55.9 ± 0.8^{a}	0.49 ± 0.01^{a}	2.79 ± 0.26^{a}

Same letters within the same column and for the same cultivar, do not statistically differ ($p<0.05$)

For instance, it has been shown that lines carrying the null allele at the *Glu-A3* did not statistically differ in dough strength from another allele when HMW-GS alleles with a positive influence on dough strength, such as subunit 5+10, were in the background (Gupta and MacRitchie, 1994). This might be one possible explanation why cv. Glenn has strong gluten strength and why cv. Lillian, CDC Plentiful and Stettler are intermediate in gluten strength. In this sense, previous studies reported that for R_{max} (maximum dough resistance) the alleles of *Glu-A3* where ranked in the following order b>d>e>c (Gupta et al., 1991; Metakovsky et al., 1990).

6.4. Conclusion

The SDS-PAGE technique was used to identify and characterize variants of the gluten protein complex (HMW-GS and LMW-GS and gliadins) in wheat cultivars with contrasting grain characteristics. The cultivars had significant effect on the baking parameters studied. Addition of L-cys (50% of allowed concentration) showed positive improvements in baking properties of dough from Glenn as it broke down the size of the glutenin aggregates. Whereas, the addition of L-cys negatively affected the baking properties of cultivars like Stettler with weak gluten strength as the size of the glutenin aggregates was reduced. Addition of L-cys to flour with strong gluten strength improved dough handling characteristics and bread quality (loaf volume and crumb firmness). Addition of L-cys to flours with strong gluten reduced mixing time (by up to 47%), increased loaf volume (up to 9%), and elasticity of the products, all the desired characteristics needed to increase theefficiancy of the automated processes for bread products.

7. General discussions

The overall goal of this research was to investigate enzymatic alternatives to the use of chemical additives as means to create cleaner label baking products. In addition, this research aimed to provide a better understanding of the new wheat classification in Canada in relation to genotype, dough handling properties, flour quality, and baking parameters. For this purpose, a screening study of a set of twenty-five commercially grown Canadian Western spring wheat cultivars selected based on historical data on their overall dough handling and baking performance was conducted. The main goal was to evaluate their composition and dough handling properties to be further narrowed to five cultivars to test the effects of different chemical oxidizers, enzymes, and a reducing agent. As result, an evaluation on cultivar-type, additive-type, and composition and their inter-relationship on final product were assessed. Overall, the quality and baking tests showed that they were very cultivar dependent, where stronger cultivars tended to have better responses in relation to rheology and additives than weaker cultivars.

The Canadian Western Red Spring (CWRS) cultivars varied little in glutenin profile, three allelic forms for HMW-GS *Glu-B1* 7oe+8, 7+9 and 14+15 were identified with prevalence of the first two subunits. In addition, the patterns of HMW-GS *Glu-D1* did not show diversity as all the lines carried the 5+10 allele. This low variation can be related to the strict end-use quality guidelines of the CWRS to maintain consistency, which could have contributed to a reduction of genetic diversity within this class over the years (McCallum and DePauw, 2008). Similar results were presented by De Vita et al. (2007) where flours with the *Glu*B1 allele coding for protein subunits 7 + 8 resulted in high alveograph W values (indicating dough baking strength) and *P/L* ratio (dough strength/extensibility), indicating stronger dough characteristics. Saint Pierre et al. (2008) found that the subunit *Glu*D1 5+10 in hard red spring wheat was associated with increased dough strength.

In contrast, variation in wheat flour quality and final bread product can also be related to growing conditions, including environment and crop management (Finlay et al., 2007). Table 7.1 presents a comparison on quality parameters and rheology for the 2016 and 2017 crop years for the five selected cultivars examined in the majority of this study.

Table 7.1. Comparison of the 2016 and 2017 wheat crops (14% moisture basis).

	Yield (%)	Hardness (HI)	Protein (%)	Ash (%)	FN (s)	DS (%)	WG (%)	GI	GPI	ABS (%)	STA (min)	DDT (min)	MTI	|G*| (kPa)	tan δ
CDC Plentiful															
2016	72.7	60	13.8	0.37	372	4.7	39.2	91.8	0.88	59.9	7.6	4.9	45	11.7	0.37
2017	72.7	74	12.1	0.31	382	4.7	36.8	96.5	0.89	67.8	8.2	4.9	42	20.3	0.34
Glenn															
2016	70.5	74	12.7	0.36	339	5.6	34.9	99.0	0.88	61.2	10.1	7.5	45	15.1	0.33
2017	71.8	78	14.0	0.29	317	3.4	35.4	98.4	0.92	58.0	10.2	7.5	42	19.1	0.32
Harvest															
2016	72.7	64	13.5	0.41	380	5.4	41.1	70.3	0.78	61.9	4.6	4.3	54	10.3	0.37
2017	72.3	81	13.1	0.33	394	4.6	40.6	84.7	0.85	60.7	5.5	4.2	53	13.7	0.33
Lillian															
2016	69.7	62	14.4	0.42	380	4.8	43.5	59.8	0.73	62.6	3.6	2.9	63	10.8	0.42
2017	71.2	68	13.4	0.38	417	3.9	40.3	78.1	0.84	61.2	4.6	3.3	57	17.6	0.35
Stettler															
2016	72.2	62	13.6	0.42	346	4.3	40.6	77.6	0.78	60.8	4.7	3.2	65	9.6	0.40
2017	72.1	71	14.1	0.31	384	4.6	43.2	90.6	0.88	59.8	7.9	4.2	40	11.6	0.35

As both crops were grown at the same location, using the same seeding rate and fertilization, variations in quality can be mostly attributed to environmental variations. During the 2017 crop season, there were 29 days of temperatures over 30 ºC, whereas 2016 had only six days (Saskatchewan Research Council, 2018). Usually, moderate-high temperatures (25–32 ºC) during the grain-filling phase could result in an increase in the gliadin to glutenin ratio, which could have a positive effect on the strengthening the dough (Wrigley et al., 1994; Borghi et al., 1995; Jarvis et al., 2008). This effect could possibly help to explain an overall increase in |G*|, for instance from 15.1 to 19.1 kPa (Glenn), 11.7 to 20.3 kPa (CDC Plentiful), and 10.8 to 17.6 kPa (Stettler) from 2016 to 2017. Higher |G*| is usually related to strong wheat cultivars and can be affected by overall gluten properties, including protein content and glutenin/gliadin ratio. However, a heat shock could result in a reduction of the size-distribution of the glutenin polymer protein (Wardlaw et al., 2002).

Even though environmental factors can not be analyzed individually, lower precipitation could explain differences in wheat flour quality and performance between 2016 and 2017. In 2017, lower monthly rain precipitation occurred in comparison to the normal precipitation, except for the month of May. In the months of June and July it was ~25 mm for each month, whereas the normal precipitation rate is ~70 and ~60 mm, respectively (Saskatchewan Research Council, 2018). As presented in Table 7.1 the 2017 crop had an overall higher protein content, gluten index, gluten performance index, GI, GPI and consequently higher dough stability, development time, and |G*| in comparison to 2016. The GI varied more for Harvest (70.3 to 84.7%), Lillian (59.8 to 78.1%) and Stettler (77.6 to 90.6%). Generally, the protein content is inversely proportional to water availability, as it favors the accumulation of nitrogen (N) of grain and lower rates of accumulation of carbohydrates (Dupont and Altenbach, 2003; Jarvis et al., 2008; Flagella et al., 2010). Triboï et al. (2003) found variations in protein fraction composition at maturity, related to post-anthesis temperature or drought, which can occur mainly because of differences in the total quantity of nitrogen accumulated during grain filling.

From the five cultivars selected for further analysis with chemical additives and enzymes, two were re-classified into a new class of wheat in 2018 (CNHR), Harvest and Lillian (Canadian Grain Commission, 2018). From the screening study, one way to conclude that Harvest and Lillian performed similarly or below the weaker wheat check for the CWRS class, Carberry (Table 3.3 and 3.4). This helps to understand the rationale for moving them from the CWRS wheat class, as it characterizes a lower range in wheat quality and strength. In addition,

PCA shows (Figure A4.5, Appendix) both cultivars forming cluster for rheology, indicating similarities and distinguishing them from the others. Loaf volume, mixing time and crumb firmness were similar for the five cultivars, differing significantly only between Glenn and Stettler. Stettler had the lowest loaf volumes and harder crumb firmness, which could be attributed to low |G*| values in addition to tan δ and J_{max}. All cultivars had a good response to chemical oxidizers, reducing agent, or enzymes, where the results were strongly cultivar-based.

Interestingly, Stettler remains in the CWRS class being one of the five most widely grown cultivars in Canada in 2017 (Canadian Wheat, 2017). However, according to Canadian Grain Commission (CGC) insured area statistics, Stettler production reduced from 7.4% to 3.4% of total area insured in 2017 crop and 2019 crop, dropping from second most produced to sixth (CGC, 2019a). The overall performance of Stettler was weaker than Carberry, Harvest, and Lillian – the weakest cultivars (Table 3.3-3.5 and 4.1-4.3). In the cultivar description from DePauw et al. (2009) Stettler had significantly (p<0.05) greater protein concentration than all of the checks except for Lillian. On the other hand, the cultivar presented lower loaf volume (1088 cm^3), mixing time (4.1 min), STA (13.5 min), and DDT (7.1 min) in comparison to the mean of checks (DePauw et al., 2009). Similar results were observed in this study, where the cultivar had the lowest oven rise and loaf volume, and highest crumb firmness (Table 5.1). In addition, Stettler had the lowest |G*| and highest J_{max} than the other four cultivars and Carberry, and lower STA, DDT, and GI than Carberry (Table 3.6), which characterize lower dough strength. On the other hand, Stettler had a similar or higher protein content, WG and hardness index than Carberry.

Not much research has been published specifically for cv. CDC Plentiful related to its quality parameters or baking performance. However, lately, this cultivar has been used as wheat baking flour check in the Grains Innovation Laboratory (University of Saskatchewan, Saskatoon, Canada). Mainly due to the cultivar's historical consistency in previous baking performance tests and grain/flour quality parameters. In addition, data from both crop years tested (2016 and 2017) classified CDC Plentiful as an intermediate-high strength cultivar overall in relation to CWRS results for flour quality (Canadian Grain Commission, 2016; 2017). In the present research, CDC plentiful demonstrated a good response in regards to additives (except for azodicarbonamide, ADA) improving bread characteristics, such as higher loaf volume, reducing mixing time and crumb firmness.

The flour additives were supplemented at 50 and 100% of the allowed concentration by Health Canada or suggested by the supplier, in the case of enzymes. The results were mostly

affected by the cultivar-type other than the additive-concentration. Harvest had a good response to ascorbic acid (AA) in improving loaf volume in addition to |G*|, which can be related to an improvement in dough strength. Similar results were also observed for glucose oxidase (Gox). This was expected as both, AA and Gox, act through oxidation. The first, oxidizing SH groups of cysteine residues in protein molecules (Nakamura and Kurata, 1997). The latter, by oxidizing d-glucose to d-gluconic acid and hydrogen peroxide (H_2O_2), which indirectly oxidizes the thiol groups of cysteine residues forming disulfide bonds (Leskovac et al., 2005). This results in improved dough handling and bread loaf properties (Bonet et al., 2007; Decamps et al., 2012; Stoica, 2013). The use of AA and enzymes for Lillian and Harvest, was effective in achieving a similar performance to the stronger cultivar Glenn, improving their overall dough properties. In addition, Lillian bread quality was positively affected by the addition of both enzymes xylanase (Xyl) and Gox, increasing loaf volume. In general, Xyl decreased |G*| but still resulted in a comparable loaf volume to other chemicals or Gox. This could be related to increased water availability from arabinoxylans hydrolyzation, which resulted in looser doughs (lower |G*|) with enough strength to maintain loaf volume.

For most cultivars a reduction of oven rise was observed at 100% concentration of ADA (i.e., Glenn, Lillian, and Harvest). This could be attributed to the fast acting characteristic of ADA and/or its over-saturation, resulting in lower loaf volume and higher crumb firmness (Table 5.1) (Sahi, 2014). However, the poorer bread performance of ADA could also be associated with the increase in dough strength as evident by the higher |G*| and lower tan δ, which would not have allowed the dough to expand during bread making (Table 4.3). In contrast, the addition of AA resulted in an overall increase in oven rise and loaf volume, and similar or reduced crumb firmness when compared to the controls. The above was associated with an increase in |G*| and lower tan δ. In addition, AA is not considered a fast-acting chemical oxidizer as is ADA. However, its action is largely completed after mixing, as it is dependent on the presence and availability of oxygen, converting it into the dehydro form, favoring the oxidation reaction (Sahi, 2014).

Enzymes performed similarly to chemical oxidizers when comparing baking properties and rheology, but in different ways. Xylanase had a tendency to reduce dough |G*| and increase tan δ. This did not impact on loaf volume negatively as values continued comparable to the control and/or Gox. Similar results were reported by Hilhorst et al. (1999) and Autio et al. (1996). A more elastic dough allows for more expansion during fermentation, which can also explain the higher loaf volume. In addition, the Xyl mechanism works to hydrolyze non-starch

components, releasing free water which changes the viscoelastic properties of dough and contributing to the final bread volume (Butt et al. 2008). The above may explain why the cultivar Glenn had a significant (p<0.05) increase in loaf volume with Xyl as it could be due to a higher protein content and increased hydration of the gluten network during dough development, fermentation, and proofing. Ahmad et al. (2014) also supported the theory that a higher loaf volume due Xyl addition is result of an increased redistribution of water from pentosans to the gluten phase, increasing the volume of the gluten fraction and, as consequence, resulting in a more extensible dough. Similar results were reported by Romanowska et al. (2003). In contrast, Gox had less of an effect on improving loaf volume and crumb firmness when compared to the control. Caballero et al. (2007) attributed the increase in volume to more elastic and cohesive crumbs caused by the supplementation of Gox. However, doughs containing Gox had a significant (p<0.05) increase in dough strength (higher |G*| and lower tan δ) in relation to the controls, but similar or equal to chemical oxidizers. Hilhorst et al. (1999) also presented lower tan δ values and a firmer dough crumb structure when using Gox compared to Xyl, which is attributed as a more elastic dough behavior. In addition, a lower concentration of Gox had a crumb softening effect in most cultivars, which was in agreement with Bonet et al. (2006).

Reducing agents have a large effect on dough rheology and final product characteristics (Song and Zheng, 2007). A significant change in tan δ (p<0.05) was found between the control and L-cysteine (L-cys) at 100% concentration for all the cultivars. It is known that L-cys reduces the average molecular weight of proteins from the thiol-disulfide interchange reaction mechanism. This effect leads to a decrease in G′ and an increase in tan δ, caused by the reduction in cross-linking in a polymer system through breaking or inhibiting the formation of disulfide links between gluten forming proteins. Thus, softening the dough structure (Lambert and Kokini, 2001; Song and Zheng, 2007). The reduction in cross-linking can also explain the reduction in mixing time by almost half compared to the controls with the supplementation with L-cys. This resulted in an overall increase in oven rise and loaf volume with 50% concentration of L-cys (except for Harvest). Glenn had smaller decrease in |G*| with the 50% concentration (from 19.1 to 15.6 kPa), whereas all other cultivars experienced an approximate 60% decrease in |G*|, e.g., from 13.7 to 5.6 kPa, Harvest. Stettler, on the other hand, had the lowest oven rise, loaf volume, and harder crumb structure.

8. Summary

Wheat has unique structure-function relationships that affect the mechanical behavior of wheat dough, due to the viscoelastic characteristics of gluten proteins (gliadins and glutenins). These characteristics influence the final product quality and consumer acceptability. Changes in wheat protein characteristics can be influenced by the environment, genetics, and crop management or grain processing factors. Any deficiencies in gluten properties (i.e., strength) that arise can typically be overcome through the addition of dough improvers (e.g., chemicals or enzymes) to guarantee and maintain wheat quality and, consequently, good dough handling and bread performance. In summary, the present research aimed to study a range of commercially grown Canadian Western Spring wheat cultivars (n = 25) as a means to characterize their properties in relation to composition and dough handling. Five cultivars having weak, intermediate, and strong dough handling characteristics were chosen to perform baking trials and dough handling tests with the addition of chemical oxidizers, reducing agent, and enzymes in different concentrations (50 and 100% of the allowed by Health Canada and suggested by the enzyme company). Therefore, it was possible to evaluate the effect of cultivars, additives, and additive concentration.

Wheat flour quality is strongly correlated to wheat properties and composition that can directly affect the final product application. Western Canadian wheat cultivars can be classified in six different classes based on their functional characteristics, where Canada Western Red Spring (CWRS) is known for delivering consistent and desirable wheat quality for breadmaking. The inter-relationship of wheat cultivars among different classes is crucial to determine wheat flour performance. For instance, Carberry and Glenn are commonly used as cultivar checks in breeding programs developing new cultivars for the CWRS wheat class. Within the cultivar range used for the present study from 2016 crop year, clusters were formed around those two specific cultivars, which made it possible to divide and define wheat strength and dough handling characteristics. Overall, cultivar type had a positive effect on proximate analyzes and directly impacted dough handling parameters. In particular protein and gluten properties impacted significantly ($p<0.05$) the dough strength parameters. Thus, strong and significant correlations were detected between gluten properties and dough strength measurements ($p<0.01$), such as micro-doughLAB and rheology, where higher stability was

observed in stronger wheat cultivars, which also conferred stronger viscoelastic characteristics in both shear rheometry and creep compliance tests. From this screening study, five wheat cultivars from the CWRS class were selected to represent varying dough strength (CDC Plentiful, Glenn, Harvest, Lillian, and Stettler).

The five cultivars selected were evaluated for their response to chemical oxidizers and enzymes treatments at different concentrations. The composition of the different crop years from the screening study and the scale up of the five cultivars (2016 and 2017) was very consistent and comparable (Table 7.1). Dough strength was more strongly affected by cultivar than additive-type and concentration, where Glenn (strongest cultivar) had the longest mixing time and stability, higher protein content and energy required to develop the dough compared to other cultivars assessed, based on mixograph, microDough-LAB and rheology. In contrast, Harvest and Lillian had weaker dough strength, requiring the lowest energy to optimal dough development and the highest mixing tolerance index. The fundamental rheology suggested that the use of the enzyme glucose oxidase (Gox) can be suitable for improving dough strength in comparison to the control and/or chemical oxidizers (ascorbic acid and azodicarbonamide), due to its improved |G*|. In contrast, the commercial fungal xylanase (Xyl) resulted in equal or poorer dough strength when compared to the control, except for Glenn where strength was improved compared to the control. The negative effect of Xyl could be related to an overdosing, releasing too much water into the dough and affecting the viscoelasticity. From the PCA analysis, clusters were formed between Harvest and Lillian, CDC Plentiful and Stettler, and Glenn suggesting they were distinct from one another. The dough handling properties are strongly correlated to bread quality, thus, baking trials help to evaluate wheat quality and their impact in the final product.

The effectiveness of different additive types and their composition evaluated for the baking parameters, such as mixing time, oven rise, loaf volume, and crumb firmness and structure, was found to be highly cultivar specific. Based on overall performance, Glenn was superior to other cultivars, regardless of the additive or additive concentration. In contrast Stettler, considered to be an intermediate strength cultivar, had poorer overall performance in relation to other cultivars, with bread loaves having the lowest volumes, oven rise and mixing time. The addition of Xyl had a positive effect on flours with medium gluten strength, through improvement in loaf volume and crumb firmness, which were softer in comparison to the control and other chemical oxidizers. Gox, on the other hand, had an overall similar performance in comparison to the control. However, it had a lower oven rise and longer mixing

time, regardless at the concentration and cultivar in comparison to the control. A slight reduction in mixing time was observed with the addition of ADA in comparison to other additives, regardless of the concentration, which could be related to its fast-acting characteristics. The AA showed good performance in oven rise and, consequently, equal or higher loaf volume in comparison to the control. All the baking performance parameters were analyzed by PCA, which showed clustering of Harvest and Stettler versus CDC Plentiful and Lillian, and Glenn suggesting they were distinct from one another. Even though cultivar was observed to have a fundamental effect on the results in relation to the oxidizers and enzymes used, more research should be performed to determine the optimal concentration for each cultivar to assess the cost efficiency of its use in substitution of oxidizers, as the impact of each additive is cultivar-specific.

In contrast to the oxidizing agents, a reducing agent (L-cysteine) was used to evaluate the baking performance and dough handling characteristics of the five wheat cultivars. In addition, the SDS-PAGE technique was used to identify and characterize variants of gliadins and HMW- and LMW-GS glutenin to identify and differentiate the wheat cultivars. Improvements in baking parameters were observed with the addition of L-cysteine at the 50% concentration, especially, for Glenn, the stronger cultivar. In addition, intermediate cultivars (CDC Plentiful and Lillian) exhibited less of a response to L-cysteine. For instance, the addition of L-cysteine negatively affected weaker cultivars like Stettler and Harvest. Therefore, L-cysteine may be a suitable additive to strong flours, improving bread quality, such as increasing loaf volume (up to 9%) and softening the crumb, and reducing dough mixing time (by up to 47%) and elasticity of the products in the case of the stronger cultivars.

The use of alternative additive to chemical oxidizers, such as glucose oxidase and fungal xylanase, to improve dough strength and baking performance was positive when compared to the controls (no additive) and chemical oxidizers. Thus, enzymes can be used to produce clean label wheat flours guaranteeing their overall performance in baking. Furthermore, this research demonstrated a significant of cultivar on the results, suggesting that cultivars blends could also be an efficient alternative to mimic similar results to chemical oxidizers or reducing agents and should be further investigated.

9. Future studies

Findings from this work showed that stronger dough cultivars were more effective at controlling dough rheology than some of the weaker varieties eliminating the need for additives. However, in the future it would be interesting to probe the microstructure of the doughs prepared from the 25 cultivars further using nuclear magnetic resonance and Fourier Transform Infrared spectroscopy with attenuated total reflectance to look at changes to the protein structure during dough development. This will allow for a better understanding of the driving mechanisms. This information could also shed some light on how the different protein profiles, especially in the weaker varieties impact network formation. Further work in this area could include a deeper examination of the impact of Gox and Xyl on water mobility within the dough network, as free water can have a big impact on dough handling and stickiness. It is hypothesized that Gox would promote gluten network development as protein-protein interactions would increase, allowing for more water to be bound. In contrast, it is hypothesized that Xyl would increase free water in the dough as it acts to hydrolyze pentosans. Possible enzyme blends between fungal xylanase (Xyl) and glucose oxidase (Gox), which have different mechanism of action, could be tested to modify the functionality of the wheat flour. Xyl action makes more water available in the media, which can favor the Gox oxidation mechanism and more protein cross-linking. Also, the concentration of the enzymes could be optimized based on dough microstructure and further tested performing baking trials. The bread making method used in the present research (Canadian Short Process, CSP) also had a very rich formulation (including whey protein, shortening, and malt) which could have masked some of the results. Because of that, the lean no time (LNT) baking test, described by Canadian Grain Commission, is suggested to be used in the future as it minimizes the use of ingredients that in their improving effect can mask inherent dough strength.

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11. Authors' Contribution

Tozatti, P., Hopkins, E.J., Briggs, C., Pierre Hucl, P., and Nickerson, M.T. (2019). Effect of chemical oxidizers and enzymatic treatments on dough rheology. *Journal of Cereal Science*, 90, 14 July 2019: 102806. https://doi.org/10.1016/j.jcs.2019.102806.

Tozatti, P., Hopkins, E. J., Briggs, C., Hucl, P. Nickerson, M.T. (in press). Effect of chemical oxidizers and enzymatic treatments on the baking quality of doughs formulated with five Canadian spring wheat cultivars. Food Science and Technology International.

Tozatti, P. designed and performed the experiments and processed the experimental data with the supervision of Nickerson, M.T. In addition, Tozatti, P. drafted the manuscript and designed the figures with support from Hopkins, E., Briggs, C., Hucl, P., and Nickerson, M.T. Hucl, P. also provided the seeds used to perform the experiments and with the space to carry out the flour milling and quality analysis. In addition, Briggs, C. was responsible for training in methods and supervision during their realization in the Crop Science Lab.

Tozatti, P., Fleitas, M.C., Briggs, C., Hucl, P., Chibbar, R.N., Nickerson, M.T. (2019). Effect of L-cysteine on the rheology and baking quality of doughs formulated with flour from five contrasting Canada spring wheat cultivars. *Cereal Chemistry*, 00, 13 November 2019: 1–13. https ://doi.org/10.1002/cche.10239.

Tozatti, P. designed and performed the experiments and analyzed the data with the supervision of Nickerson, M.T. Fleitas, M.C. carried out the SDS-Page experiment with the support of Tozatti, P. Briggs, C. was responsible for providing baking training and bread analyzing data. Hucl, P. provided the seeds to perform the experiments and helped interpret the results in relation to his data collection. In addition, Tozatti, P. wrote the manuscript with support from Fleitas, M.C., Chibbar, R.N., Nickerson, M.T., and Hucl, P.

12. Appendix

Table A3.1. Significant ($p<0.05$ and $p<0.01$) correlation analysis for flour quality and dough rheology properties.

	MDT	PKH	ETP	SAP	BTW	TEG	ABS	STA	DDT	MIT	ASH	PROT	LIP	HI	FN	WG	DG	GI	JMAX	JR	JEL	TAND	|G*|
MDT	1																						
PKH	-0.13	1																					
ETP	0.91**	0.19	1																				
SAP	0.24*	-0.57**	0.00	1																			
BTW	-0.09	-0.12	-0.16	0.23	1																		
TEG	-0.02	0.82**	0.27*	-0.37**	0.01	1																	
ABS	-0.41**	0.42**	-0.26*	-0.31**	-0.15	0.24*	1																
STA	0.74**	0.06	0.74**	0.14	0.27*	0.15	-0.32**	1															
DDT	0.72**	0.12	0.72**	0.13	0.22	0.17	-0.20	0.87**	1														
MIT	-0.61**	0.06	-0.61**	-0.19	-0.24*	-0,08	0.25*	-0.87**	-0.69**	1													
Ash	-0.16	0.08	-0.16	-0.03	-0.06	0.08	0.14	-0.33**	-0.44**	0.28*	1												
Prot	-0.35**	0.61**	-0.07	-0.47**	-0.03	0.46**	0.61**	-0.06	-0.04	0.02	0.07	1											
Lip	0.17	-0.21	0.04	0.21	-0.06	-0.22	-0.05	0.26*	0.24*	-0.31**	-0.26*	-0.25*	1										
HI	-0.13	0.01	-0.21	0.01	0.06	-0.01	0.41**	-0.29*	-0.19	0.29*	0.31**	-0.13	-0.02	1									
FN	-0.18	0.17	-0.10	-0.30**	0.20	0.11	0.01	0.00	-0.13	-0.02	0.38**	0.20	-0.07	0,02	1								
WG	-0.57**	0.50**	-0.35**	-0.46**	-0.06	0.34**	0.69**	-0.28*	-0.25*	0.21	0.12	0.89**	-0.12	-0.05	0.21	1							
DG	-0.38**	0.50**	-0.14	-0.44**	-0.01	0.39**	0.56**	-0.06	-0.06	0.02	0,041	.880**	-0,034	-0,103	0,199	.918**	1						
GI	0.68**	-0.02	0.68**	0.12	0.15	0.06	-0.47**	0.65**	0.69**	-0.62**	-0.45**	-0.23*	0.09	-0.30**	-0.21	-0.48**	-0.30**	1					
Jmax	-0.64**	0.41**	-0.47**	-0.33**	-0.12	0.28*	0.64**	-0.47**	-0.48**	0.44**	0.35**	0.65**	-0.19	0.11	0.18	0.78**	0.66**	-0.67**	1				
Jr	-0.67**	0.44**	-0.49**	-0.36**	-0.07	0.29*	0.62**	-0.46**	-0.48**	0.45**	0.32**	0.65**	-0.21	0.08	0.20	0.79**	0.67**	-0.62**	0.96**	1			
Jel	0.11	-0.19	0.02	0.14	0.22	-0.18	-0.35**	0.10	0.08	-0.08	-0.12	-0.36**	0.01	-0.01	-0.01	-0.35**	-0.30**	0.26*	-0.47**	-0.25*	1		
TanD	-0.61**	0.30**	-0.44**	-0.37**	-0.34**	0,18	0.51**	-0.61**	-0.58**	0.55**	0.33**	0.61**	-0.23*	-0.04	0.10	0.72**	0.58**	-0.63**	0.83**	0.82**	-0.32**	1	
|G*|	0.59**	-0.54**	0.35**	0.44**	0,09	-0.40**	-0.72**	0.38**	0.35**	-0.32**	-0.17	-0.77**	0.21	-0.04	-0.15	-0.83**	-0.73**	0.42**	-0.83**	-0.87**	0.30**	-0.72**	1

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed)

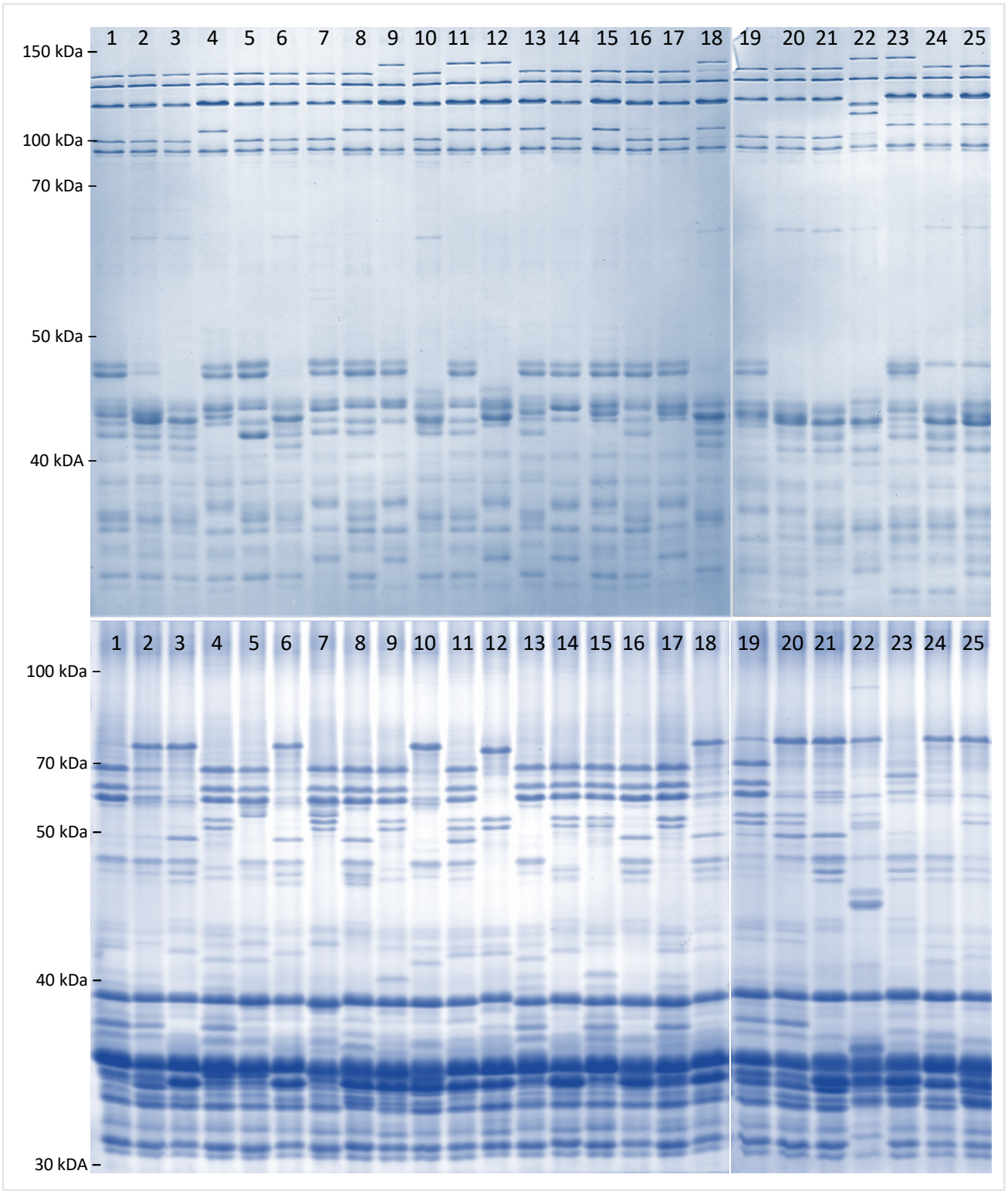

Figure A3.1. SDS-PAGE of the 25 wheat varieties showing glutenins (top) and gliadins (bottom) protein banding patterns. From left to right 1: Harvest, 2: Pembina, 3: McKenzie, 4: Roblin, 5: Glenn, 6: Unity VB, 7: Carberry, 8: Lillian, 9: CDC Utmost, 10: CDC Stanley, 11: CDC Plentiful, 12: CDC Titanium, 13: Shaw VB, 14: Stettler, 15: AAC Redwater, 16: AAC Brandon, 17: Parata, 18: Whitehawk, 19: AAC Iceberg, 20: CDC Whitewood, 21: CDC Kinley, 22: Pasteur, 23: CDC Terrain, 24: 5702PR and 25: CDC Walrus.

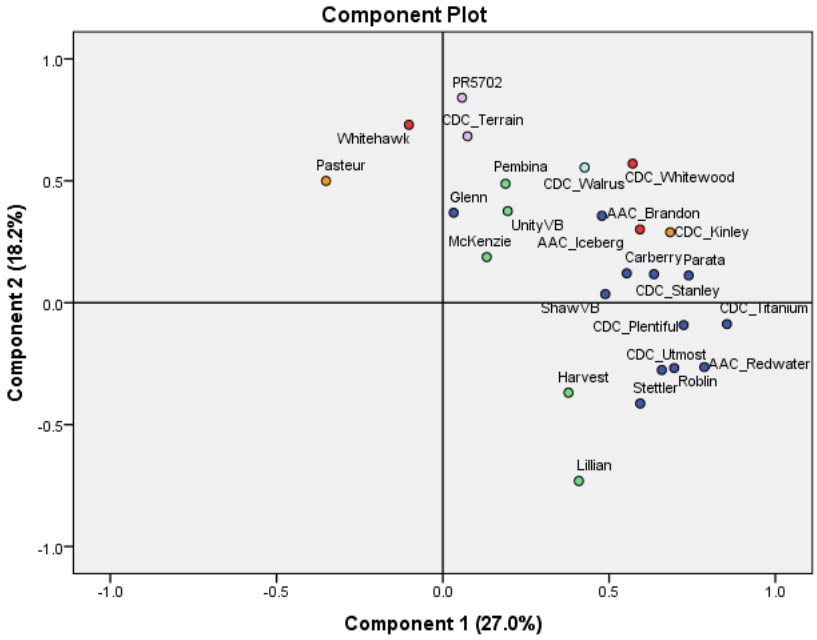

Figure A3.2. Principal component analysis of compositional data. Different colors represent different wheat classes. Green, CNHR; blue, CWRS; yellow, CWSP; red, CWHWS; and light blue CWES.

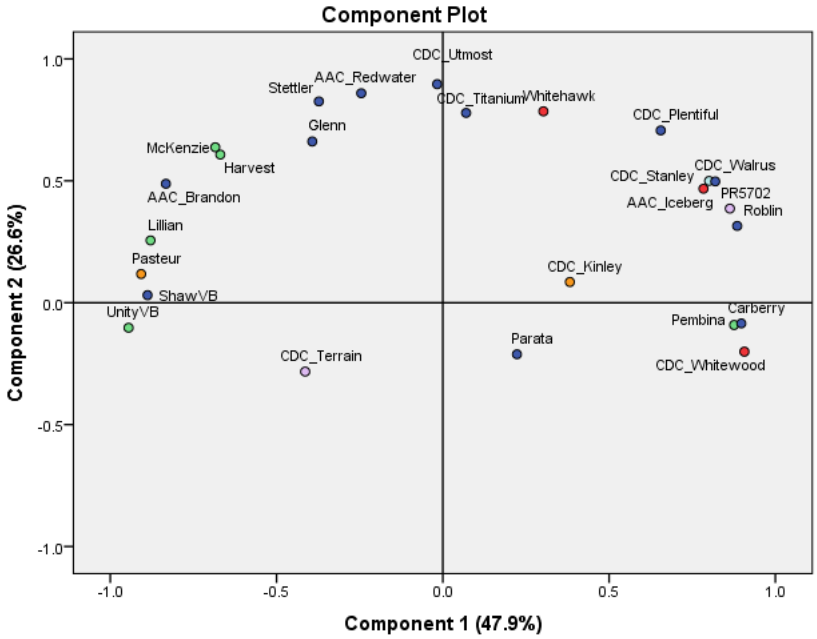

Figure A3.3. Principal component analysis of solvent retention capacity data. Different colors represent different wheat classes. Green, CNHR; blue, CWRS; yellow, CWSP; red, CWHWS; and light blue CWES.

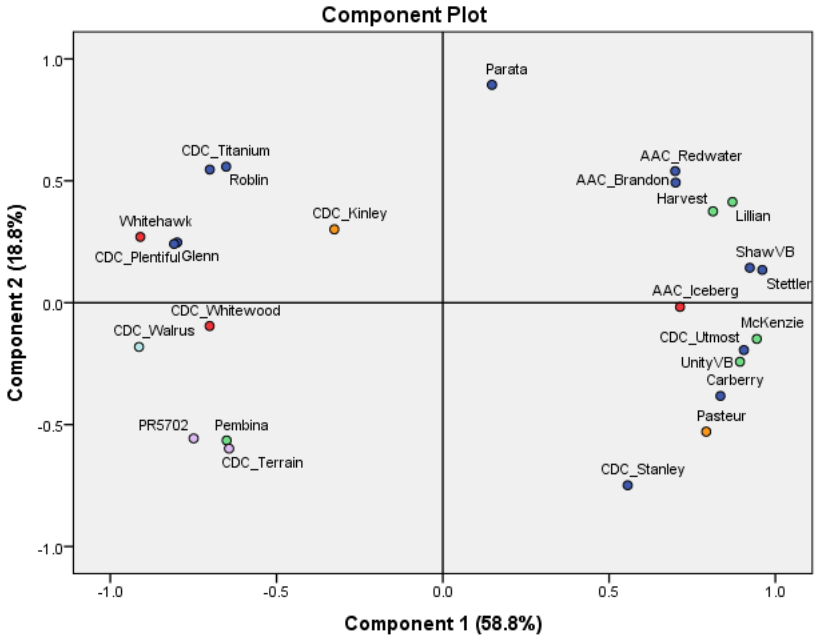

Figure A3.4. Principal component analysis of micro-doughLab data. Different colors represent different wheat classes. Green, CNHR; blue, CWRS; yellow, CWSP; red, CWHWS; and light blue CWES.

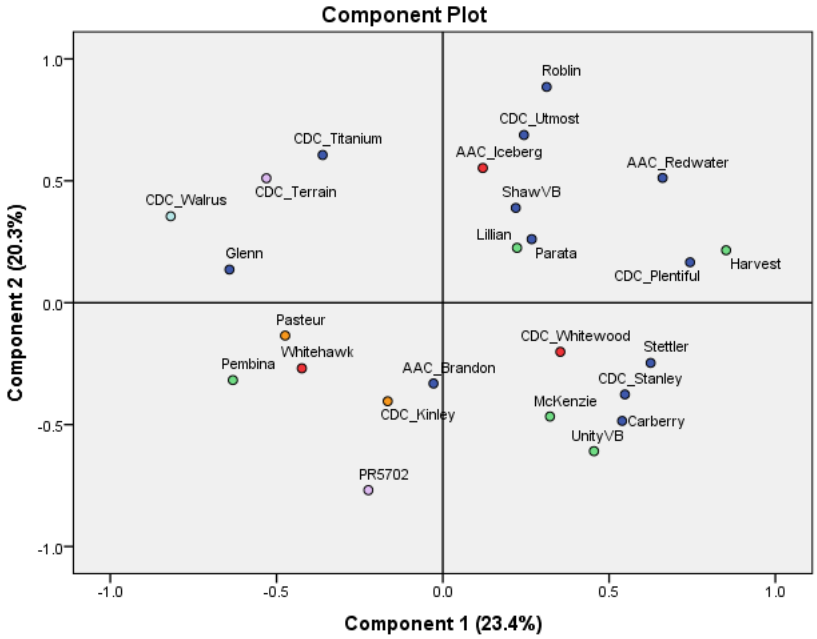

Figure A3.5. Principal component analysis of mixograph data. Different colors represent different wheat classes. Green, CNHR; blue, CWRS; yellow, CWSP; red, CWHWS; and light blue CWES.

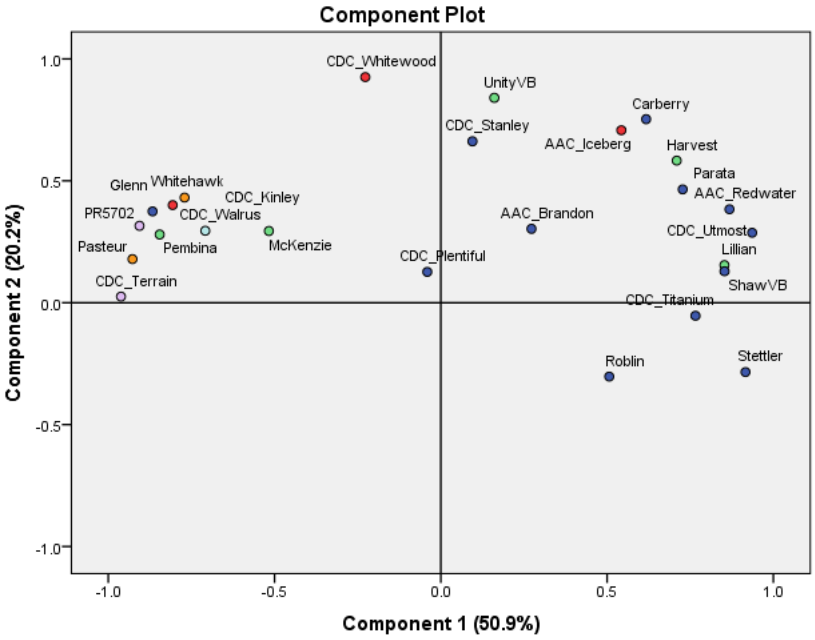

Figure A3.6. Principal component analysis of rheology data. Different colors represent different wheat classes. Green, CNHR; blue, CWRS; yellow, CWSP; red, CWHWS; and light blue CWES.

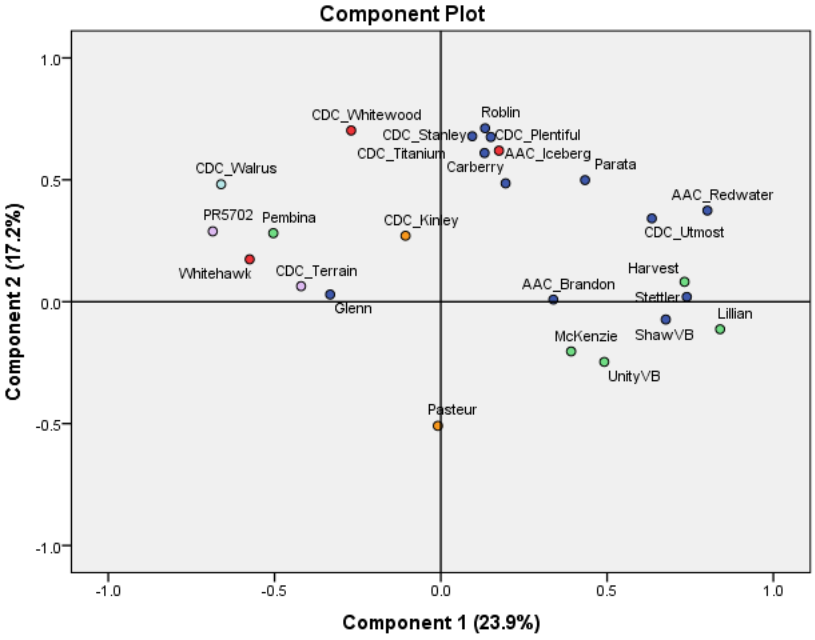

Figure A3.7. Principal component analysis of all parameters analyzed. Different colors represent different wheat classes. Green, CNHR; blue, CWRS; yellow, CWSP; red, CWHWS; and light blue, CWES.

Table A4.1. Analysis of variance output for rheology data in relation to cultivar, concentration, and additive-type.

Independent variables	Dependent variables			
	|G*|	tan δ	J_{max}	J_{el}
a) Main effects				
Cultivar	$p<0.001$	$p<0.001$	NS	NS
Treatment	$p<0.001$	$p<0.001$	NS	NS
Concentration	$p<0.01$	NS	NS	NS
b) Two-way interactions				
Cultivar × additive	$p<0.001$	$p<0.001$	NS	NS
Cultivar × concentration	$p<0.01$	NS	NS	NS
Additive × concentration	$p<0.001$	$p<0.001$	NS	NS
c) Three-way interaction				
Cultivar × additive × concentration	$p<0.001$	$p<0.05$	NS	NS

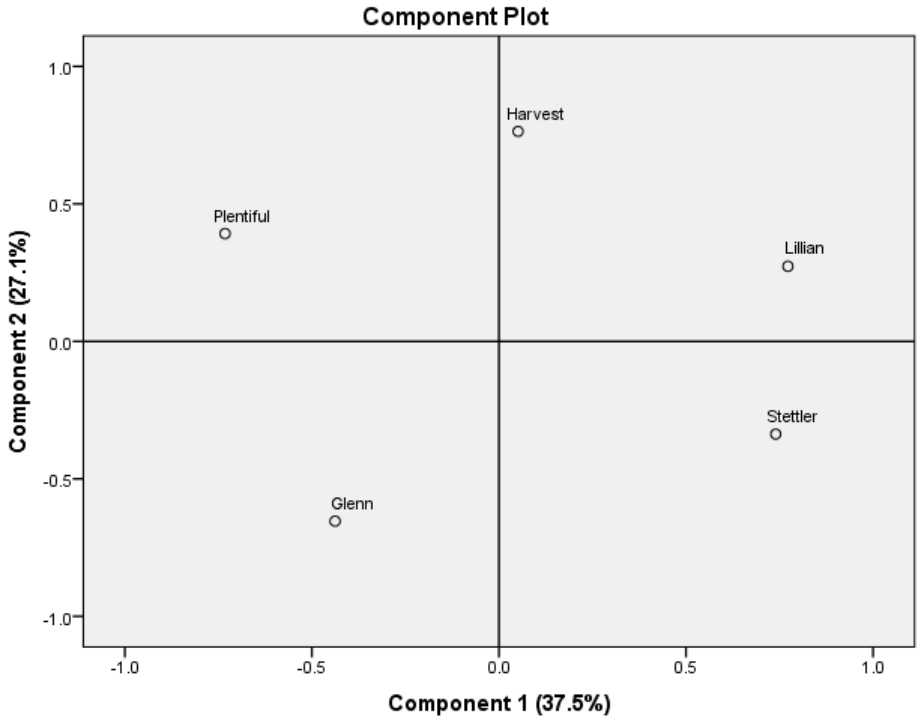

Figure A4.1. Principle component analysis of compositional data.

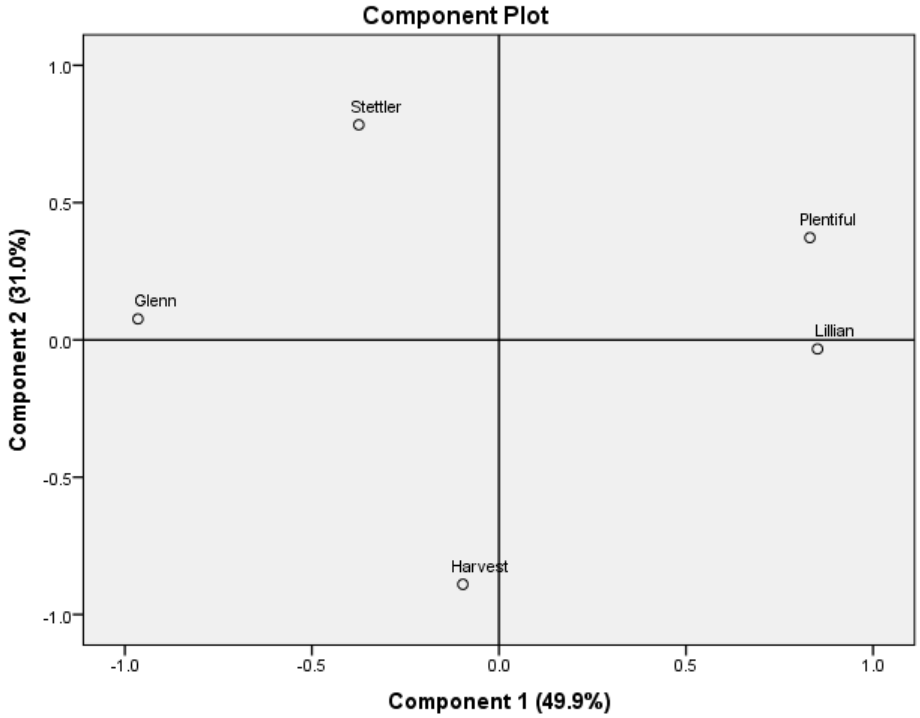

Figure A4.2. Principle component analysis of solvent retention capacity data.

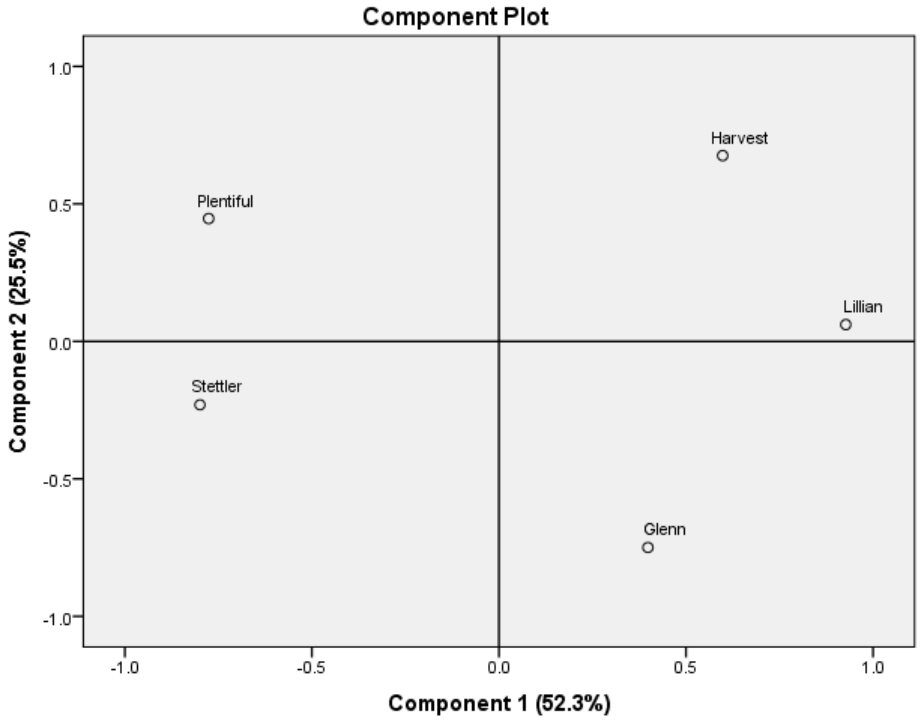

Figure A4.3. Principle component analysis of mixograph data.

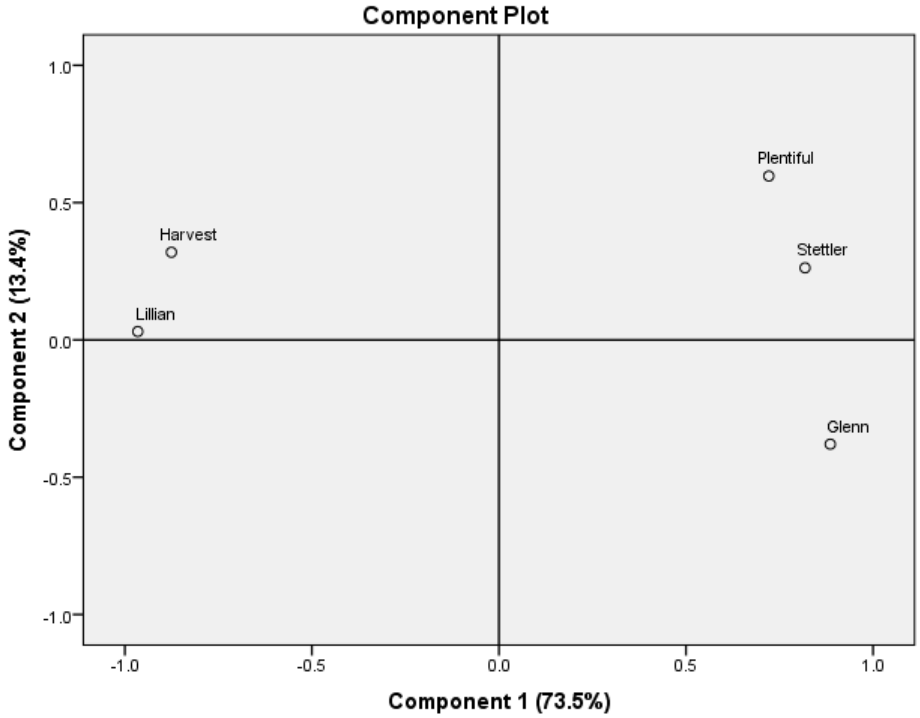

Figure A4.4. Principle component analysis of micro-dough lab data.

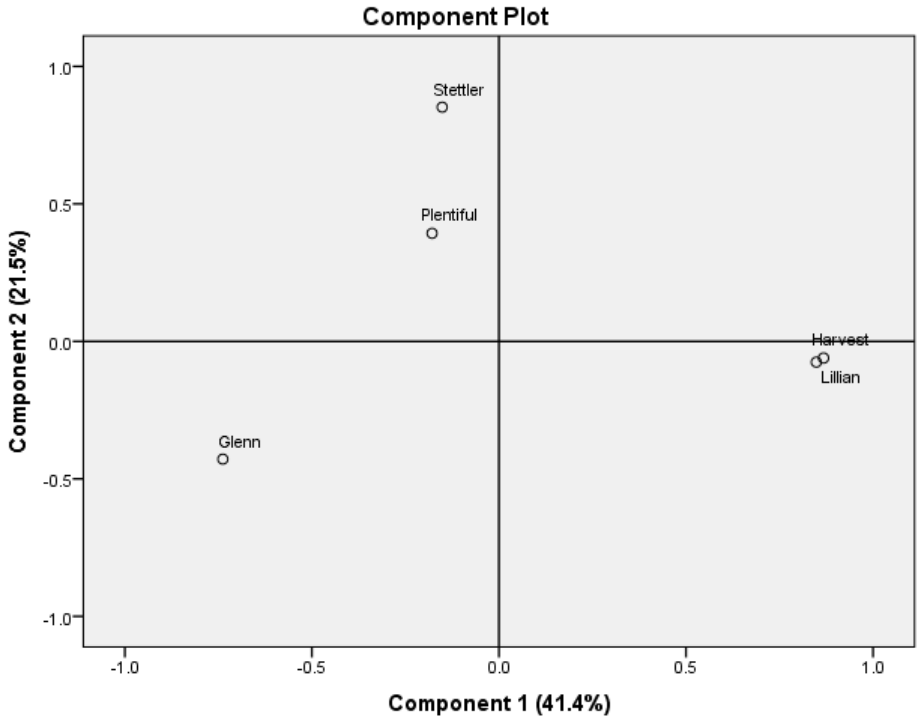

Figure A4.5. Principle component analysis of rheological data.

Table A5.1. Analysis of variance output for baking property measurements.

Independent variables	**Dependent variables**			
	Mix time (min)	**Oven rise (cm)**	**Loaf volume (cm^3)**	**Crumb Firmness (Force, g)**
I) Chemical Oxidizers and Enzymes				
a) Main effects				
Cultivar	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
Additive	$p<0.05$	$p<0.001$	$p<0.001$	$p<0.001$
Concentration	NS	NS	$p<0.001$	$p<0.05$
b) Two-way interactions				
Cultivar x additive	NS	$p<0.01$	$p<0.001$	NS
Cultivar x concentration	NS	NS	$p<0.05$	NS
Additive x concentration	NS	$p<0.05$	$p<0.001$	$p<0.05$
c) Three-way interaction				
Cultivar x additive x concentration	NS	NS	$p<0.001$	$p<0.05$

Table A5.2. Analysis of variance output for C-cell property measurements.

Independent variables	**Dependent variables**						
	Slice Area	**Slice Brightness**	**Cell Contrast**	**Number of Cells**	**Area of Cells**	**Cell Wall Thickness**	**Cell Diameter**
I) Chemical Oxidizers and Enzymes							
a) Main effects							
Cultivar	$p<0.001$	$p<0.05$	NS	$p<0.001$	$p<0.05$	$p<0.05$	$p<0.05$
Additive	$p<0.001$	$p<0.01$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$
Concentration	$p<0.001$	NS	NS	NS	$p<0.05$	NS	NS
b) Two-way interactions							
Cultivar x additive	$p<0.001$	NS	NS	$p<0.05$	NS	NS	$p<0.01$
Cultivar x concentration	NS	NS	NS	NS	NS	NS	NS
Additive x concentration	$p<0.001$	NS	NS	$p<0.05$	$p<0.05$	NS	NS
c) Three-way interaction							
Cultivar x additive x concentration	$p<0.001$	NS	NS	NS	NS	NS	NS

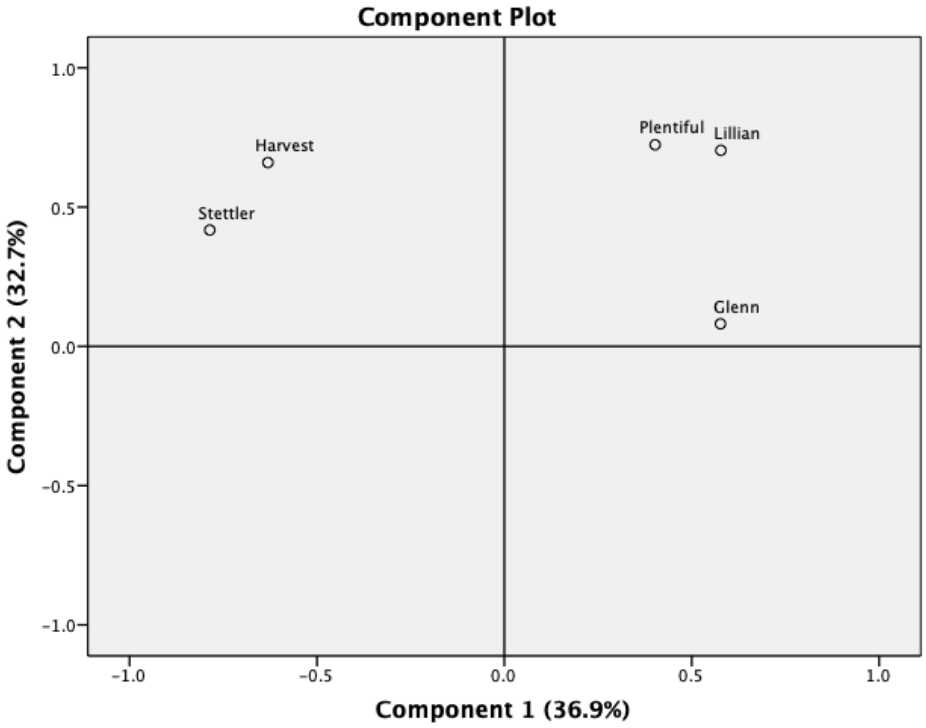

Figure A5.1. Principle component analysis for baking parameters.

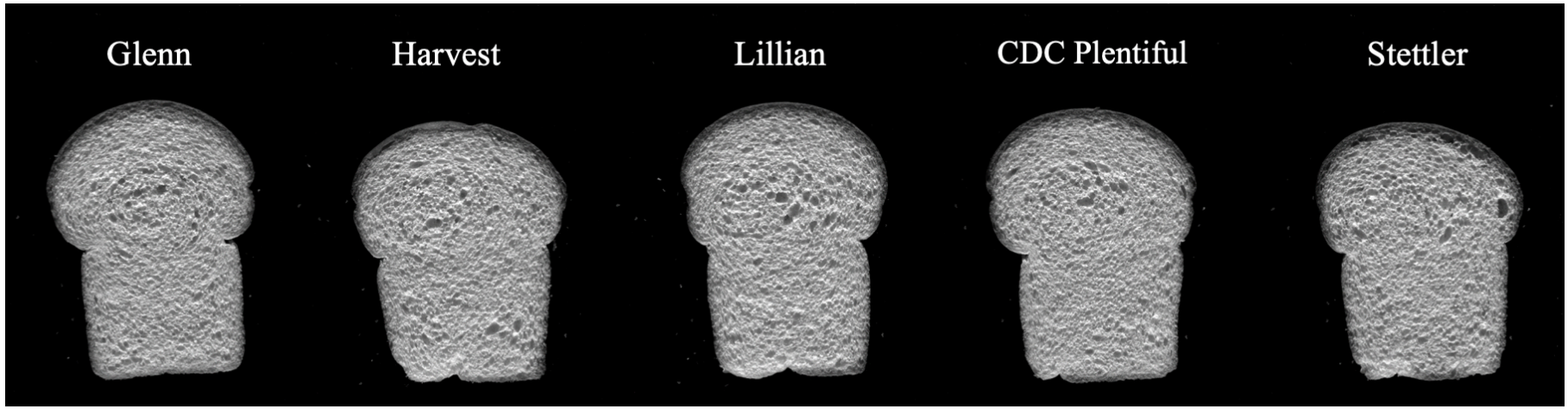

Figure A5.2. C-cell imaging of bread baked using different wheat cultivars. Loaves contain no additives.

Table A6.1. Analysis of variance output for C-cell property data.

Independent variables	Dependent variables						
	Slice Area (mm^2)	**Slice Brightness (-)**	**Cell Contrast (-)**	**Number of Cells (-)**	**Area of Cells (%)**	**Cell Wall Thickness (mm)**	**Cell Diameter (mm)**
Reducing Agent and Control							
a) Main effects							
Cultivar	$p<0.001$	$p<0.05$	NS	$p<0.001$	NS	NS	NS
Concentration	$p<0.001$	$p<0.01$	NS	$p<0.001$	$p<0.05$	$p<0.01$	$p<0.01$
b) Two-way interaction							
Cultivar × Concentration	NS	NS	NS	NS	NS	NS	NS

Table A6.2. Pearson correlations between bread-making parameters and C-cell analysis of five Canadian spring wheat cultivars (2017 crop year).

	Mix Time (min)	Oven Rise (cm)	Loaf Volume (cm^3)	Firmness (gF)	Slice Area (mm^2)	Slice Brightness	Cell Contrast	# Cells	Area of Cells (%)	Wall Thickness (mm)	Cell Diam (mm)
Mix Time (min)	1										
Oven Rise (cm)	0.41*	1									
Loaf Volume (cm^3)	0.49**	0.91**	1								
Firmness (gF)	-0.41*	-0.73**	-0.88**	1							
Slice Area (mm^2)	0.49**	0.91**	0.98**	-0.85**	1						
Slice Brightness	0.42*	0.54**	0.49**	-0.49**	0.51**	1					
Cell Contrast	0.31	0.37*	0.33	-0.26	0.31	0.63**	1				
Number of Cells	0.55**	0.82**	0.85**	-0.74**	0.84**	0.73**	0.60**	1			
Area of Cells (%)	-0.45*	-0.08	-0.01	-0.06	-0.01	-0.66**	-0.75**	-0.39*	1		
Wall Thickness (mm)	-0.54**	-0.55**	-0.56**	0.53**	-0.53**	-0.75**	-0.69**	-0.88**	0.62**	1	
Cell Diameter (mm)	-0.63**	-0.32	-0.33	0.30	-0.31	-0.70**	-0.77**	-0.68**	0.85**	0.88**	1

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table A6.3. Pearson correlations between bread-making parameters and shear rheology of five Canadian spring wheat cultivars (2017 crop year).

	Mix Time	**Oven Rise**	**Loaf Volume**	**Crumb Firmness**	**TanD**	**|G*|**	**J_{max}**	**J_{el}**
Mix Time	1							
Oven Rise	0.41*	1						
Loaf Volume	0.49**	0.91**	1					
Crumb Frimness	-0.41*	-0.73**	-0.89**	1				
TanD	-0.38*	-0.51**	-0.54**	0.47**	1			
|G*|	0.46**	0.51**	0.55**	-0.41*	-0.88**	1		
J_{max}	-0.19	-0.29	-0.30	0.18	0.75**	-0.69**	1	
J_{el}	0.38*	0.43*	0.50**	-0.39*	-0.83**	0.78**	-0.91**	1

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).